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International Conference on Ultraviolet Carcinogenesis

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Arthur C. Upton, *Director, National Cancer Institute*

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INTERNATIONAL CONFERENCE ON ULTRAVIOLET CARCINOGENESIS

A Conference

held in

Warrenton, Virginia

March 21–23, 1977

Sponsors:
Division of Cancer Cause
and Prevention
and the
Cancer Biology Program,
Frederick Cancer Research Center
National Cancer Institute

Editors:
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Introduction

The need for holding a meeting on ultraviolet carcinogenesis was recognized more than three years ago by Drs. John Spikes and Frederick Urbach. It was clear, even then, that a summary of the current investigations in this field was needed. Work related to ultraviolet carcinogenesis encompasses a broad spectrum of disciplines, ranging from cellular biochemistry to cancer epidemiology, and an international conference seemed an appropriate mechanism for defining the state of the art. Through the cooperation of many individuals, this meeting has at last come about under the joint sponsorship of the Division of Cancer Cause and Prevention of the National Cancer Institute and the Cancer Biology Program of the Frederick Cancer Research Center.

In addition to summarizing current research, this conference is intended to provide an opportunity to discuss problems of common interest from rather diverse points of view. With this goal in mind, we have tried to cover a wide range of research topics. On behalf of the organizing committee, I would like to thank all the participants for contributing to what promises to be an informative and timely meeting.

Margaret L. Kripke

SESSION I

Experimental Ultraviolet Carcinogenesis

Chairman: Frederick Urbach

Co-Chairman: Harold F. Blum

Welcome and Introduction:

Evidence and Epidemiology of Ultraviolet-Induced Cancers in Man[1, 2]

Frederick Urbach[3]

To open formally this International Conference on Ultraviolet Carcinogenesis is indeed a privilege. I should like to begin with a historical note: Exactly 13 years ago, to the day, on March 21, 1964, a conference on "Sunlight and Skin Cancer" was held here at Airlie House. Abstracts of the papers of this conference are appended to this volume. That conference also was sponsored by the National Cancer Institute on the suggestion and urging of Professor Harold Blum, one of its founding members. A group of 13 investigators assembled that spring day in 1964, and in the next 5 days discussed many of the same problems that we will discuss in the next few days in the spring of 1977. The proceedings of that conference were only mimeographed in abstract form: There was not enough money to get them published! The proceedings apparently were so anonymous, that 7 or 8 years later, the Library of the National Cancer Institute had to call me to find out where a copy could be found because numerous requests for reprints had arrived, and the Library had no knowledge of where to find them!

Of the 13 members of that conference, 4 are here today: Professors Blum, Latarjet, Magnus, and myself. Rudolph Schulze and Harold Silverstone have died. One, Donald Robertson, is still actively engaged in the study of photocarcinogenesis, and the remaining members have turned to other pursuits.

I believe that these comments are of interest. That conference in 1964 really was the first international conference on ultraviolet carcinogenesis. The first meeting of the Comité Internationale de la Lumière, the forerunner of the present Association Internationale de la Photobiologie, was held in Lausanne in 1928, and its main theme was "Light-Induced Skin Cancer." In September 1978, on the 50th anniversary of its first congress, the Association will meet again in Lausanne to discuss this problem. So there was a hiatus of 36 years between the first discussion on light and skin cancer and the second and a lapse of 13 years from 1964 to 1977.

At the first conference on "Sunlight and Skin Cancer" in 1964, many interesting problems were discussed and many recommendations were made that are now considered prophetic. One was that the knowledge of skin cancer epidemiology was woefully lacking then; it is only a little less woefully lacking now. We discussed the pathology of skin tumors, and we will hear much more about that this week. At that meeting, Robertson and Latarjet first spoke about their integrating UV-B meters, and you will hear about much better and more accurate instruments from Mr. Berger and about personal UV dosimeters from Dr. Ian Magnus. The conference participants discussed at great length and recommended what laboratory studies should be done. Again, it was prophetic that of paramount importance were dose–rate and intensity-effect studies, and that immunologic effects could be important; even DNA repair was mentioned!

Each conference has little sidelights that members like to remember because they leaven the work and provide a light diversion. Such an incident happened here in March 1964. If you look out the side windows of the magnificent Airlie House dining hall, you will see a small formal garden, with statues and a view of the road and the lake.

One bright, sunny March day, our group stood in that garden, chatting after lunch and admiring the scenery. While we were chatting, a fox appeared, crossed the road, hopped on a fence rail, ran along a few feet, and took off. While we were commenting on the unspoiled beauty of the Virginia countryside and the wildlife, we heard the baying of hounds. In short order, a pack of hounds arrived, followed by the local hunt dressed formally in red coats and bowlers, to the sound of hunting horns. To the amazement of our confrères from abroad, the huntsman in his top hat rode up to us and said: "Did you see which way the fox went?" Don Robertson was the only one who had the presence of mind to answer: "Yes, he went *that* way!" (pointing in the direction opposite to that which the fox had taken). The huntsman thanked him gravely and with a toot of horn and much yelping and yapping, the pack and the hunt took off in the wrong direction! I really cannot remember exactly which one of our guests shook his head in unbelief, but I shall always remember the words: "Only in the United States . . .!"

With this as background, let me briefly review with you the evidence for the hypothesis that skin cancer in man is causally related to chronic solar UV radiation exposure. At first, we need to acquaint ourselves with the world distribution of UV radiation, particularly that considered to be carcinogenically effective, the UV-B (280–320 nm). For the 1964 conference, Professor Rudolph Schulze calculated the distribution of the biologically most effective, solar UV-B radiation (approximately 280–320 nm) over the globe (text-fig. 1). He used Bener's (*1*) figures for the solar UV-B spectrum for the various zenith angles, prepared an effectiveness spectrum (text-fig. 2) taking into account the then accepted erythema action

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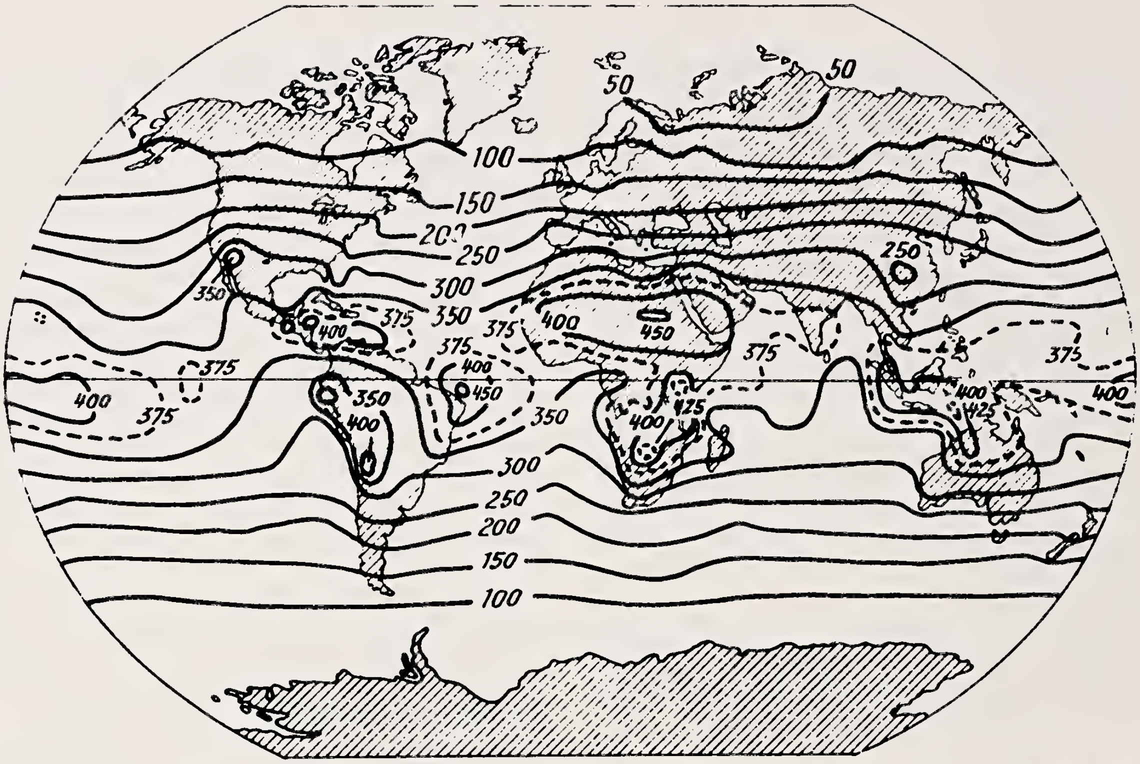
Abbreviations: UVR = ultraviolet radiation; UV-B = approximate radiation of 280–320 nm.

[1] Presented at the International Conference on Ultraviolet Carcinogenesis, held in Warrenton, Virginia, March 21–23, 1977.

[2] Supported by Public Health Service grant ES00269 from the National Institute of Environmental Health Sciences.

[3] The Center for Photobiology, Skin and Cancer Hospital, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140.

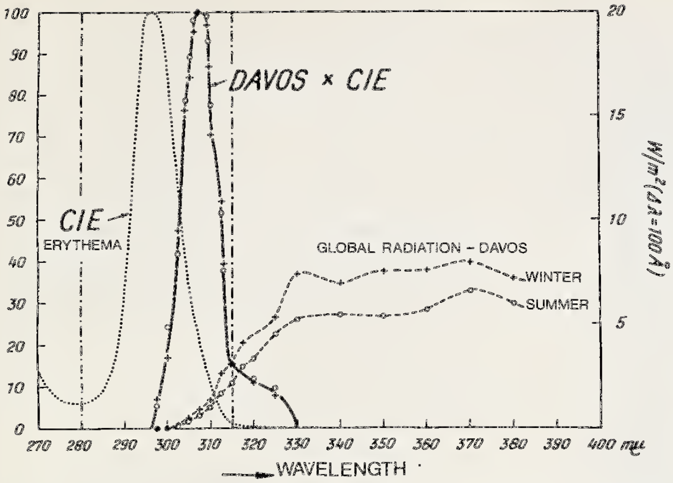


TEXT-FIGURE 1.—Distribution of annual solar UVR (307.5 nm) worldwide according to Schulze's calculations *(2)*, corrected for altitude, average ozone concentration, and cloud cover.

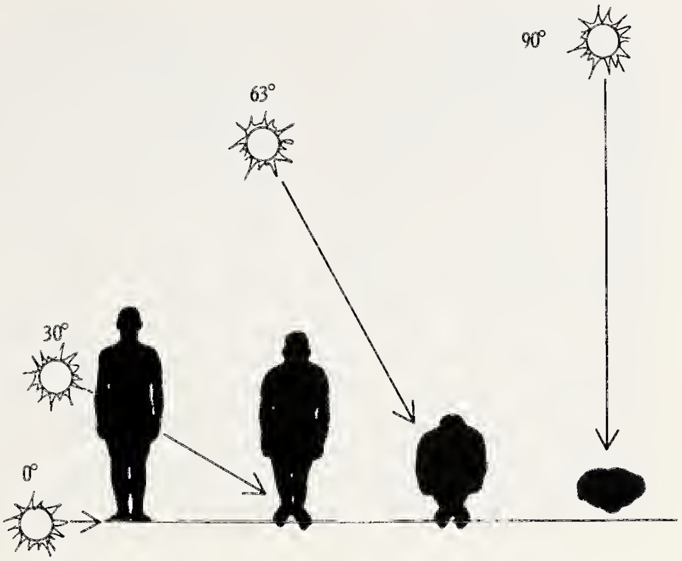
spectrum (*2*), and plotted this erythemally weighted UV-B radiation for clear days. These plots were most instructive; the effective UV-B radiation is not by any means only latitude dependent. Local differences having to do with average ozone profiles, altitude, etc., markedly modify the UV-B doses. Since Schulze's pioneer maps, many elegant calculations have been performed (*3*) with recent ozone data, weighting for cloud cover, aerosol, etc., and actual measurements have been made (*4*). Schulze's data have only been slightly improved or altered in detail. His approach, concept, and execution of an extremely difficult mathematical problem survives intact as a milestone in the field of photobiology.

The major link in the chain of evidence associating human skin cancer with exposure to UV-B radiation is found in the latitude distribution of nonmelanoma skin cancer. Plotted in text-figure 3 are incidence figures obtained in four places in the United States by the Third National Cancer Survey Team in 1972 (upper bar) and from a variety of other sources the world over (lower bar) against latitude (*5*). Clearly, there is a striking inverse relationship of nonmelanoma skin cancer incidence and latitude and, since latitude is linearly related to UV-B dose, that relationship holds for UVR as well. Next the distribution of skin cancers of the head and neck with skin areas receiving the most erythemogenic UV-B (fig. 1) is striking. The incidence–dose relationship is almost perfect for squamous cell carcinoma, and two-thirds or more of basal cell carcinomas occur on the most heavily exposed skin sites (*6*). Purely geometric reasons for the distribution of skin cancer sites incriminate UV-B. The areas of the upright human body most exposed when the sun's zenith angle varies from 0° to 40° are the top of the scalp, ears (if not covered by hair), nose, cheeks, lower lip, the shoulders, and upper back as illustrated in text-fig. 4 (*7*). Only when the sun is lower in the horizon and little UV-B is received because of atmospheric path lengths is the rest of the body irradiated. Again, these geometric patterns fit the known distribution of skin cancers.

Another part of the story has to do with the genetics of melanin pigmentation, a major absorber of UV-B radiation in human skin. It has long been known that individuals with significant genetic melanin pigmentation (blacks, Japanese, Chinese, Indians, Thai's, etc.) rarely develop skin cancer (*8*).



TEXT-FIGURE 2.—"Effectiveness spectrum" for solar UVR. Multiplying the output of the light source (the sun) by the relative effectiveness of the radiation for the production of erythema (*curve* CIE: erythema action spectrum) results in the solid line effectiveness spectrum that peaks at 307.5 nm. [Reproduced with permission from Schulze *(2)*.]

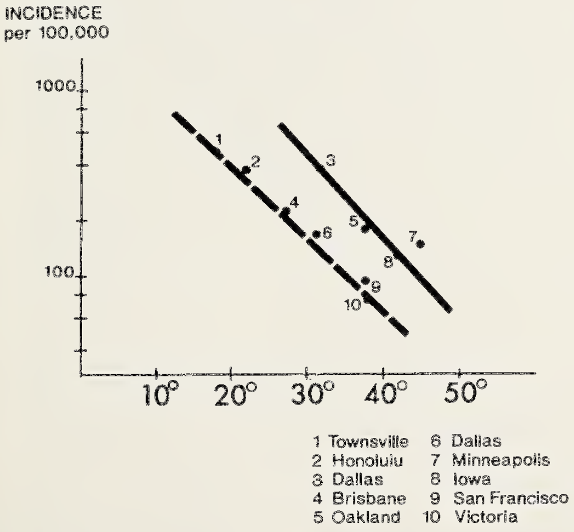


TEXT-FIGURE 4.—Surface area of direct sunlight exposure on a man standing facing the sun at differing solar elevations. [Reproduced with permission from *(7)*.]

Furthermore, people carrying dominant genetic traits for freckling and an incomplete melanin screen (mostly Celts: Irish, Scotch, and Welsh), who tan irregularly, have the highest incidence of skin cancer. This association (table 1) has been clearly demonstrated in Ireland, Australia, and the United States (*6*).

R. Setlow recently summarized the crazy quilt of factors that have to be considered if one wishes to understand the etiologic agents interacting in skin carcinogenesis (table 2). Among other factors of significance are those that can be grouped under the heading of "life-style" (occupation, time of day, and length of exposure, intervals of exposure, clothing, habits, hobbies, etc.). We are beginning to understand a little about the effect of dose fractionation and protraction in experimental carcinogenesis, and it is reasonable to believe that such factors will be of importance for human skin as well. Even aside from the uncertainty that is inherent in cell division and tumor growth, matters that Professor Blum will be addressing presently, it is clear from such an analysis why it is still extremely difficult to make any reasonable projections as to potential changes in human skin cancer incidence with possible changes in UV-B radiation exposure (*9*).

The reason for the sudden interest in photocarcinogenesis was the realization, about 5 years ago, that man's growing technology could and probably would alter the thickness of the stratospheric ozone layer (*10*). The potential effects of fleets of supersonic aircraft flying in the stratosphere became a problem suddenly discussed in public about that time, and since then further concern has arisen about the possible effects of chlorofluorocarbon release, fertilizers, bromine, etc. The list of agents with a deleterious effect on stratospheric ozone is getting longer by the day (*11*). Text-figure 5 shows the results of calculations of the effect of a 50% reduction of stratospheric ozone on the spectral distribution and intensity



TEXT-FIGURE 3.—Incidence of nonmelanoma skin cancer. *Upper line*=data of the Third National Cancer Survey *(4)*. *Lower line* =composite from various studies performed at different times in the past two decades. *Note* general parallelism of slopes of incidence relative to decreasing latitude *(10)*.

TABLE 1.—*Incidence of skin cancer by ethnic origin* [a]

Country of origin	Percent expected	Percent found
Ireland	3.45	25.5
Italy	8.3	11.0
Germany	3.9	9.5
Russia	5.0	7.7
England	4.6	6.9

[a] Data were derived from 325 patients living in Philadelphia 1958–1960 (1960 census). The relative proportion of Celtic individuals in any group of patients with nomelanoma skin cancer is significantly greater than in a matched control group. [Reproduced with permission from *(6)*.]

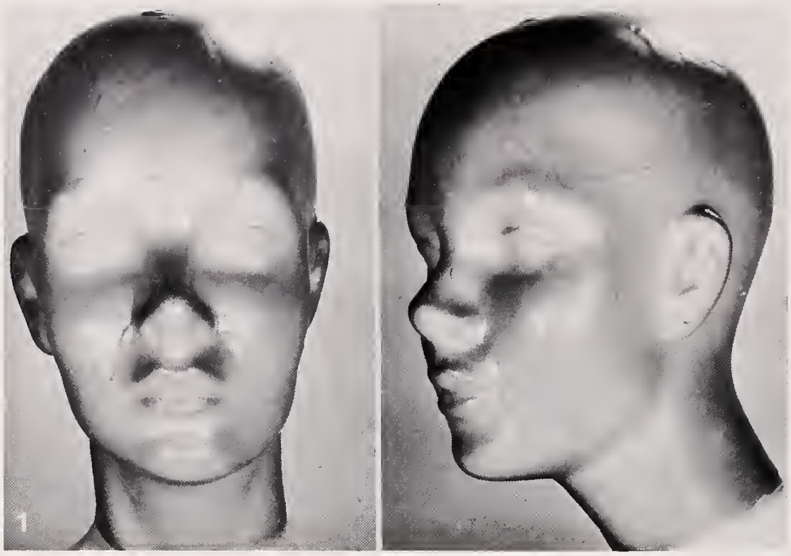
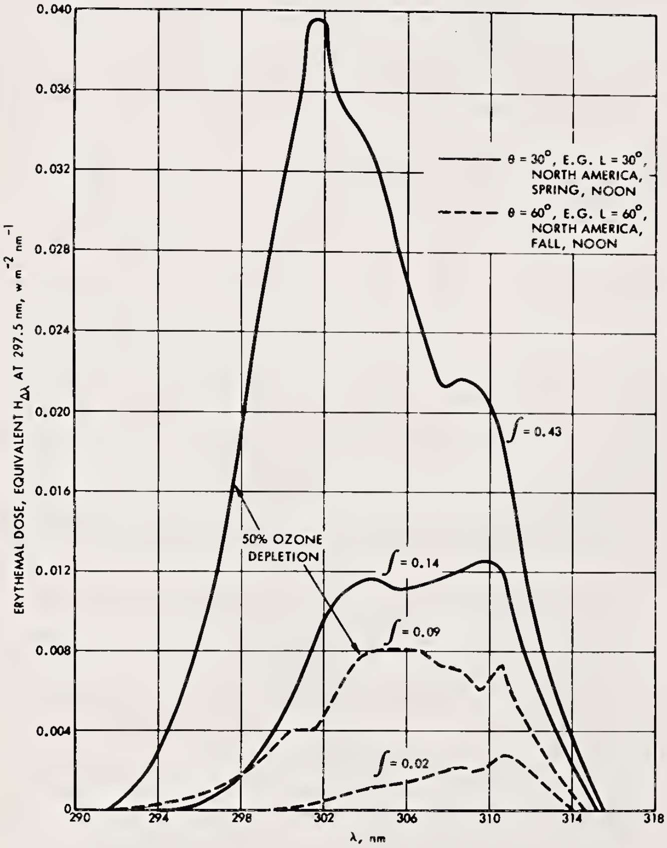


FIGURE 1.—Mannequin head coated with photosensitized wax mixture, with an action spectrum similar to human skin erythema and exposed to solar UVR. *Note* darkening of areas most exposed to erythemogenic UVR. [Reproduced with permission from *(6)*.]

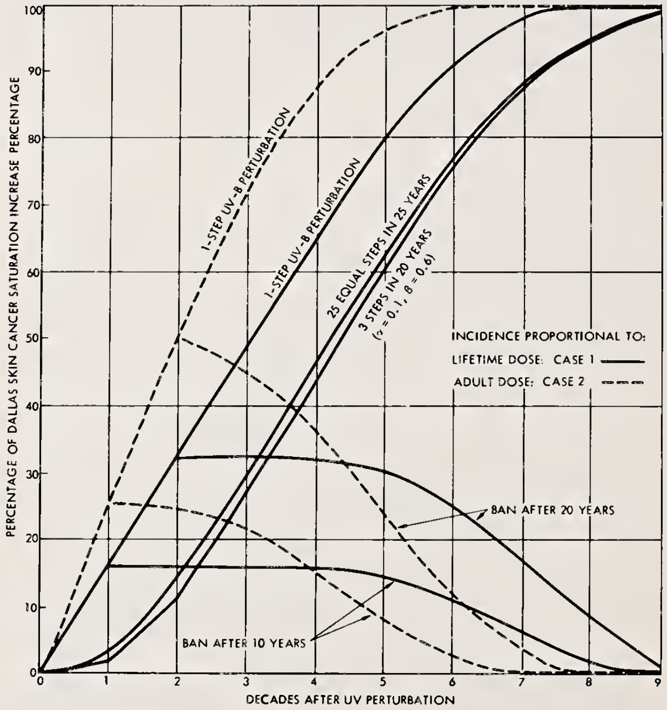
of solar erythema effective UV-B radiation. Note not only a striking increase in intensity but also a significant shift of both peak and lower limits in the direction of shorter wavelengths. The importance of such a spectral shift for skin photocarcinogenesis has only recently been recognized, and Dr. Forbes will discuss his findings later. Of course, a 50% reduction in stratospheric ozone would not long be a problem for man; most plants would probably die long before people, and we would not have to worry about an increase in the incidence of skin cancer, since most of the population-at-risk would soon starve to death.

However, changes of about 10–15% reduction in ozone are entirely possible, and we should be concerned about their effect. In an analysis of a series of models, the following fairly represents the state of the art.

We are certain of the relationship of the thickness of the stratospheric ozone layer to the amount of solar UV-B that



TEXT-FIGURE 5.—Effect of 50% ozone depletion on erythemal dose. *Note* total increase in dose, as well as shift of peak effective wavelength (from 308 to 302 nm).



TEXT-FIGURE 6.—Growth of skin cancer incidence for Dallas white males and recovery curves following a hypothetical ban on stratospheric flight.

TABLE 2.—*Some variables in sunlight-induced skin cancer*[a]

Life-style	Sampling	Environmental
UVR averaged over the yr	Age	Wind
Time of day at which exposure begins	Genetic background	Visible light
Time duration/exposure	Occupation	Temperature
No. of exposures/yr		Humidity, etc

[a] Variables are those to be considered in any attempt to correlate UVR exposure to incidence of skin cancer.

TABLE 3.—*Effect of reduction on the stratospheric ozone layer on the incidence of skin cancer*[a]

Factor	Numerical value	Range
Physical amplification	2	1.4–2.5
Biologic "	2	0.5–3.5
Total "	4	0.7–8.76

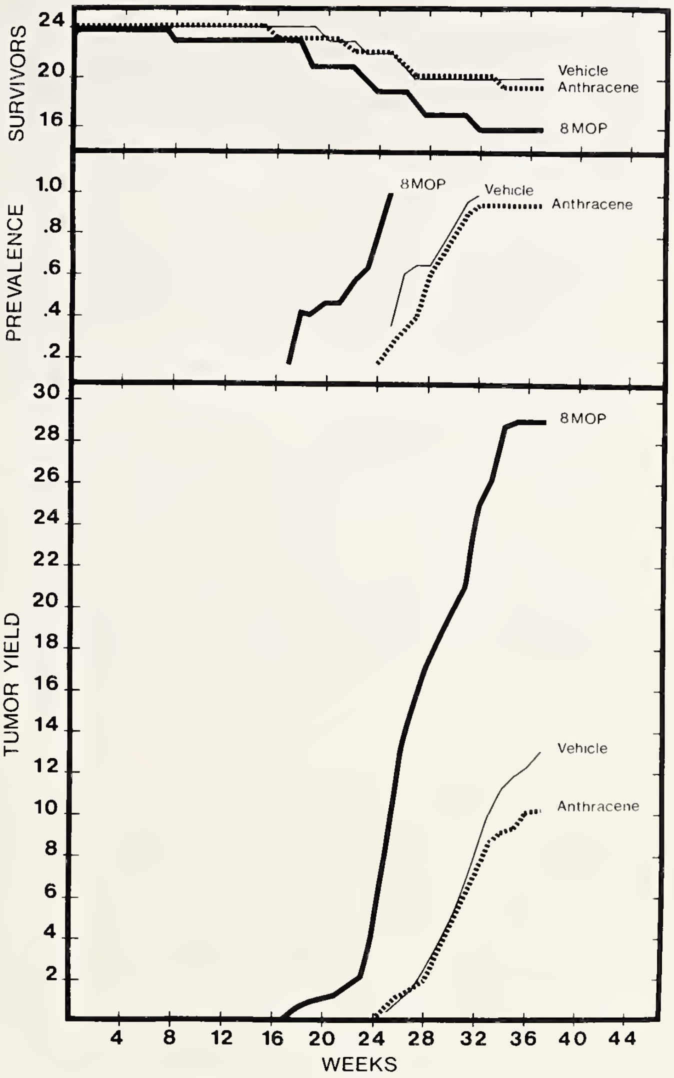
[a] Values are most recent estimates of factors relating stratospheric ozone decreases to nonmelanoma skin cancer increases (applicable to conditions in the United States for which models were developed).

can reach earth. Since the ozone absorption characteristics are well-known, and good models exist for UV scattering and absorption by air molecules and aerosols, the data are reliable, with little probable error. Thus the "physical amplification factor" (O_3 to UV-B) is approximately 2. That is, for each 1% reduction in ozone, there is approximately a 2% increase in solar erythema-effective UV-B. This will vary a little from north to south. Much more difficult to estimate is the "biologic amplification factor," i.e., the relationship of any increase in erythema-effective UV-B to increase in the incidence of skin cancer. The latest projections (and their uncertainties) are shown in table 3 (*11*).

Of course, the regulating agencies would like to have a projection from ozone decrease to skin cancer increase. Because of the huge uncertainties inherent in the data base (e.g., the true incidence of skin cancer by *effective received UV dose*), which underlies estimates of the biologic amplification factor, such projections are simply not feasible at this time.

Finally, even if such projections were feasible, they would represent the state of a new equilibrium. The changes in ozone concentration will occur gradually over several decades. Skin cancer, a disease of older people, is caused by a cumulative effect of UVR over a lifetime. Dr. Cutchis has shown this graphically in text-figure 6. With a new ozone equilibrium reached in decades, the new skin cancer incidence would not come to equilibrium for 60 to 100 years (*12*). The return to an old steady state would be even longer. Thus anything done to the ozone layer now will reflect itself only in the next generations in terms of changed skin cancer incidence.

All the data and the extrapolations from such data are based on the additivity of the relative effectiveness of different wavelengths. This is a simple assumption, which almost certainly is *not* true, and will have to be explored seriously in the future.



TEXT-FIGURE 7.—Effect of pretreatment with 8-methoxypsoralen (▬▬), anthracene (. . . .), or the vehicle (methy alcohol) (———) on the survival, the proportion of mice with tumors at any given time (prevalence), and the average number of tumors per survivor (tumor yield) in groups of albino SK-1 mice irradiated chromically/hr with a xenon arc solar simulator. [Reproduced with permission from *(13)*.]

Finally, there is increasing concern about interactions of UVR and the effect of the increasing number of photoactive chemical agents we emit into our environment. At this time, we cannot predict whether a phototoxic agent will be photocarcinogenic, nor do we know how to test for such an effect. For instance, in text-figure 7 are shown the photocarcinogenic effects of two phototoxic agents, 8-methoxypsoralen and anthracene. Both cause marked erythema, redness, and injury to the skin of mice and men when exposed to the radiation from a solar simulator in an acute experiment (*13*). On chronic irradiation, 8-methoxypsoralen augments photocarcinogenesis, whereas anthracene does not. The interactions of many such environmental photoactive agents will have to be studied. At this time, it is not possible to predict from an acute experiment whether a phototoxic agent will be photocarcinogenic or not.

Having raised many questions and given a few answers, I shall now turn the conference over to the participants, who certainly represent the most informed and experienced scientists in this important branch of photobiology.

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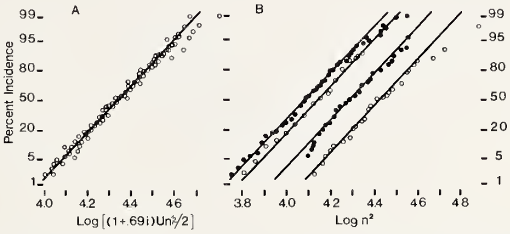
Ultraviolet Radiation and Skin Cancer in Mice and Men: Accumulation of Effect and Uncertainty of Prediction[1]

Harold F. Blum[2]

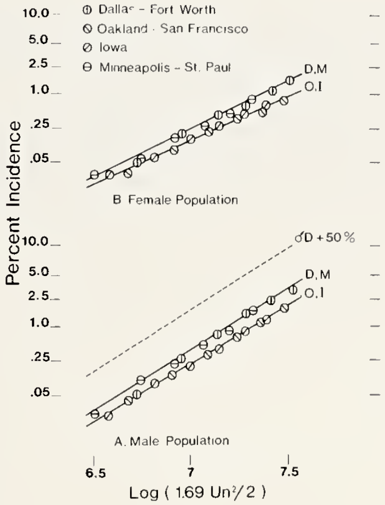
ABSTRACT—Studies on mice exposed to repeated doses of UVR (35 yr ago) and recent epidemiologic investigations and measurements of carcinogenic radiation on patients with nonmelanoma skin cancer showed that repeated doses accumulated. The effects of factors such as genetics, human behavior, kind of skin, variation in intensity of sunlight, etc., cannot be evaluated quantitatively because of uncertainty. The possibility of inherent indeterminacy must be kept in mind in the study of effects of sunlight.—Natl Cancer Inst Monogr 50: 11–12, 1978.



TEXT-FIGURE 1.—Data from experiments on cancer induced in mice by UVR. A) Data from 4 experiments and others brought together by appropriate correction. B) Data from 4 experiments. *See (1).*

It is 35 years since Hugh Grady, John Kirby-Smith, and I performed a long series of experiments at the National Cancer Institute on induction of cancers in mice with repeated doses of UV radiation. The experiments proved that the effects of repeated doses accumulate; this accumulation was expressed in terms of the square of the number of doses. The data, summarized in text-figure 1, are plotted on probability paper. At B, incidence of cancers is plotted against the logarithm of the square of number of doses. The points fall along straight lines, showing that the effects accumulate.

Cancers grow by an increase in the cell population and the rate of cell duplication. These two factors cannot be measured simultaneously, so we face uncertainty. By assigning equal weight to each of these factors, I combined the data as in text-figure 1 at A, where the points fall along the same line. This probabilistic dodge permits analysis of the data quantitatively but does not resolve the uncertainty. We are faced with indeterminacy, which may be compared with Heisenberg's indeterminacy regarding the position and direction of an electron within an atom.



TEXT-FIGURE 2.—Incidence of nonmelanoma skin cancer in man plotted on a probability grid. *Curve* D + 50% describes estimated increase of skin cancer in the male population of Dallas-Fort Worth area if there were an increase of 50% in UVR. *See (1).*

Within the last few years, epidemiologic studies and measurements of the carcinogenic radiation in sunlight have shown that the effects of repeated exposures to sunlight accumulate. In text-figure 2, the data for nonmelanoma skin cancer for four regions of the United States are plotted in the same way as the data for mice. The points fall along parallel straight lines. However, the lines do not follow the expected order with regard to latitude, measured sunlight, or sex, and cannot be brought to the same line as the data for mice in text-figure 1A. We again face uncertainty, which is better shown by the plotting in text-figure 3, in which the stippled area indicates uncertainty of current predictions about sunlight and skin cancer. More measurements that cover a wider range of latitude may reduce the uncertainty but cannot eliminate it from our predictions.

Melanoma skin cancer is distributed more erratically (text-fig. 4). This is to be expected, since sunlight can be only an exacerbating factor in melanomas.

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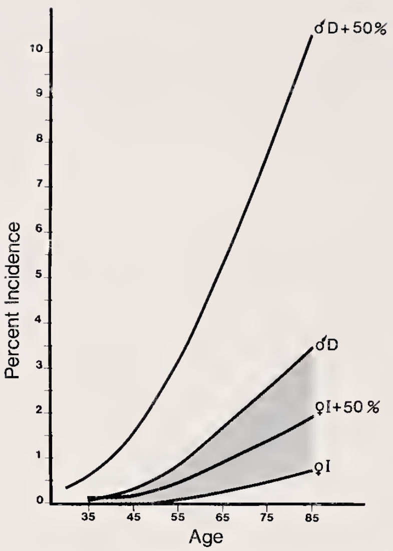
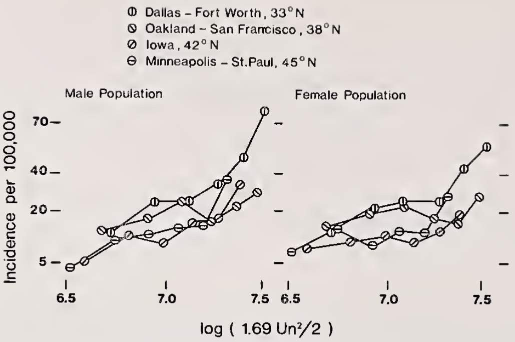
[1] Presented at the International Conference on Ultraviolet Carcinogenesis, held in Warrenton, Virginia, March 21–23, 1977.

[2] The Center for Photobiology, Skin and Cancer Hospital, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140.



TEXT-FIGURE 3.—Uncertainty of prediction. *Stippled* area indicates uncertainty of prediction regarding nonmelanoma skin cancer as shown by existing data. *Curves* ♂ D and ♀ I represent, respectively, the incidence in males of the Dallas-Fort Worth area and in females in Iowa. Curves ♂ D = 50% and ♀ I + 50% represent expected incidences if there were a 50% increase in carcinogenic UVR.



TEXT-FIGURE 4.—Incidence of melanoma skin cancer according to latitute. *See (1).*

Various factors, such as genetics, human behavior, kind of skin, variation of intensity of sunlight, etc., could affect the position of the curves in text-figure 2 but cannot be evaluated quantitatively because of uncertainty.

These studies on skin cancer cannot be directly applied to other aspects of the sunlight factor in ecology. It would seem that most effects of sunlight on plants and animals are not cumulative; however, in others they may be. For example in plankton, it has been shown that sunlight may kill organisms and also decrease the rate of reproduction (*2*).

The possibility of inherent indeterminacy should be kept in mind in the study of all effects of sunlight.

Analysis of these data and additional references can be found in (*1*).

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(*2*) CALKINS J, NACTWEY S: UV effects on bacteria, algae, protozoa, and aquatic invertebrates. *In* Impacts of Climatic Change (Climatic Impact Assessment Program Monogr 5). Springfield, Va.: Natl Tech Inform Serv, 1975, pp 3–30

Photocarcinogenesis: A Review[1, 2]

John H. Epstein[3]

ABSTRACT—Clinical observations and epidemiologic studies indicate that the sun is the primary stimulus for most human skin cancer formation. However, investigations directly confirming this association as well as defining the action spectra, time–dose relationships, energy level requirements, etc., have been confined to animal experimentation. Studies in which gross methods are used indicate that the experimental carcinogenic action spectrum falls primarily between 280 and 320 nm. Quantitative studies and tumor promotion investigations indicate that UV-induced cancer formation begins with the initial exposure. Heat, wind, and moisture stimulate UV carcinogenesis. Also, exogenous chemicals may influence carcinogenesis as photosensitizers such as 8-MOP, as additive carcinogens as noted with DMBA, or as promoters as described for Croton oil, retinoic acid, and BCNU. Qualitative studies indicate that progressive alterations occur in the epidermal–dermal basement membrane and dermal connective tissue and mucopolysaccharides associated with the progressive development of epidermal cancers. Malignant melanomas have also been induced experimentally in hairless mice with UV energy. Mechanistically, immunologic alterations and effects on DNA have received the most attention. Tumor-specific antigenicity as well as antigen deletion has been demonstrated. Immune suppression by antilymphocyte serum and certain chemicals has led to stimulation of tumor development. Perhaps the most exciting new information relates to the demonstration that chronically UV-irradiated mice have not rejected highly antigenic UV-induced cancers. This indicated that UV irradiation specifically altered the immunologic responses of the animals to these tumors. Within recent years, the influence of DNA injury and repair on cutaneous carcinogenesis has received a great deal of attention. This has been partly due to the demonstration of defective repair of UV-induced DNA damage in patients with XP. The primary photosensitive problem in these patients is an inordinate sensitivity to the carcinogenic effects of sunlight. However, correlation of DNA injury and repair directly with cancer formation has not been accomplished.—Natl Cancer Inst Monogr 50: 13–25, 1978.

Cutaneous carcinomas are by far the most common of all human cancers. In addition, more information concerning the etiology of these tumors is available than for cancers of other organs. Extensive astute observations strongly suggest that sunlight plays a significant role in the production of the vast majority of these growths. However, investigations directly confirming this association as well as defining energy levels, action spectra, time–dose relationships, etc., have been confined to animal experimentation, not only because of the impropriety of such studies in humans but also because of their impracticality. Tumor induction in human skin would most likely require 10 to 20 years or longer of repetitive, closely monitored exposures.

This review is not designed to be exhaustive in nature. The reader will be supplied with almost complete historical information concerning UV carcinogenesis in (*1–5*). This discussion is concerned with the past as it might relate to some of the information that comprises the present and perhaps the future.

ACTION SPECTRUM

In any photobiologic reaction, determination of the action spectrum generally receives the highest priority. In the realm of skin cancer, this has proved to be an almost insurmountable task due to a number of factors, including the numerous potential wavelengths, the considerable number of animals necessary, the extensive number of exposure times, and the prolonged period required for exposures to each wavelength at various intensities, as well as the difficulties in immobilization of the animals and the need for an especially accurate monochromatic source with minimal, stray light contamination. However, studies in which gross methods are used have established that the carcinogenic action spectrum in experimental animals extends primarily from 230 to 320 nm (*1, 6–8*). Furthermore, the rays between 280 and 320 nm appear to be more effective than the shorter UV rays. Within the past few years, the studies by Freeman and co-workers with a monochromator confirmed these findings (*9, 10*). From the available information, the primary carcinogenic rays are in the acute erythrogenic or sunburn spectrum. The actual shape of the photocarcinogenic spectrum has not been determined. Setlow (*11*) has proposed that the common denominator is the action spectrum for affecting DNA. However, no specific information correlates any specific event associated with acute UV injury with cancer formation except perhaps in the rare, inherited disease constellation XP.

In addition, the influence of other wavelengths, such as the UV-A (320–400 nm) on the response to the UV-B (280–320 nm), must be considered. UV-A have markedly accentuated the acute damage produced by UV-B (*12, 13*). Thus not only determination of the action spectrum but determination of

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Abbreviations: 8-MOP = 8-methoxypsoralen; XP = xeroderma pigmentosum; UV-A = long wavelengths of UV radiation; UV-B = carcinogenic UV rays; 20-MCA = 20-methycholanthrene; DMBA = 7,12-dimethylbenz [*a*] anthracene; BCNU = 1,3 bis(2-chloroethyl)-1-nitrosourea; BM = basement membrane; PAS = periodic acid-Schiff; H & E = hemotoxylin and eosin; MTX = methotrexate; NSS = normal saline solution.

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[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

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the effects of various components of the sun's spectrum on cancer production comprise essential subjects for further study.

QUANTITATIVE INVESTIGATIONS

Traditionally, the skin of the ears of albino mice and, to a lesser extent, albino rats, was used for experimental production of tumors by UV radiation because the ears of these animals lack the three main naturally inhibiting factors: pigment, a thick stratum corneum, and hair. The types of tumors produced in this tissue were primarily sarcomas, which were adequate for the monumental quantitative studies accomplished by Blum and his co-workers (*1*). Using genetically homogeneous (strain A) albino male mice, they demonstrated that dose–rate dependence followed the law of reciprocity when doses greater than 0.35×10^4 to 0.4×10^4 ergs/cm^2/second were used (*14*). Below this rate, UV became less efficient in producing tumors in their system.

In subsequent studies, Blum reported (*1, 15*) that within narrow limits, differences in doses, intensities, or intervals between doses did not alter the shape or slope of tumor incidence curves but only their positions on the log time axis (text-fig. 1). Blum (*1, 15*) and Rusch et al. (*16*) also noted that tumors would not appear within the lifetime of their experimental animals unless the exposures were administered for a sufficient period. Blum found that 50 exposures over 74 days was the smallest number of doses and the shortest time that would lead to cancer formation in his experimental system. Higher doses over a shorter time were ineffective.

With the accumulated data, Blum surmised that UV-induced cancer formation is a continuous cumulative process that begins with the initial exposure. He postulated that the appearance of tumors within the lifetimes of the experimental animals depends on growth acceleration by each exposure. In support of this concept, Rogers (*17*) produced adenomas in mouse lung tissue with a single exposure. In addition, Epstein and Roth (*18*) produced cancers in the skin of hairless mice with a single UV exposure followed by Croton oil promotion. Subsequently, Pound (*19*) confirmed these findings in hairy mice.

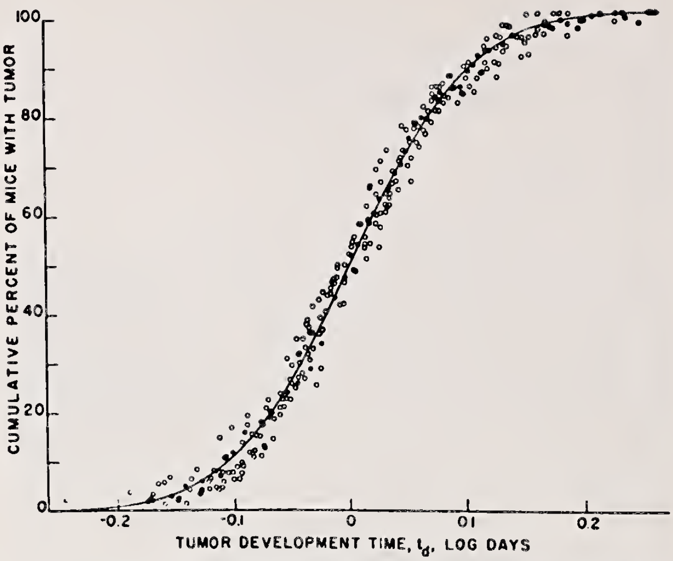
The mechanism of this acceleration of tumor growth is not clear. Production of mitosis-stimulating substances or perhaps removal of the mitosis-inhibiting materials "chalones" (*20, 21*), might have some effect.

Numerous aspects of time–dose relationships and reciprocity remain to be evaluated, including studies on dose protraction, dose fractionation within a limited period, and the limits within which total dose levels will affect tumor formation.

PHYSICAL AND CHEMICAL INFLUENCES ON PHOTOCARCINOGENESIS

Physical Influences

The enhancing effect of heat on the degree of cutaneous injury and intensity of the erythema response to UV energy is well documented (*22*). Freeman and Knox (*22*) and Bain et al. (*23*) demonstrated that increased temperatures at the time of UV exposure accelerated tumor production. High humidity and wind have also been shown to accentuate acute UV damage (*24, 25*). Owens et al. (*26*) reported that increased wind velocity accelerated UV-induced tumor formation in experimental animals. The mechanisms of these responses have not been determined.



TEXT-FIGURE 1.—Distribution of the tumor development time, t_d, in a population of genetically homogeneous (strain A) male mice exposed to UV, the exposures being placed at regular intervals up to the time of tumor appearance. Data are from 12 experiments with 676 mice. Dose and interval between doses was the same for a given experiment but varied between experiments. Mean values of t_d varied accordingly, and all points have been corrected to a common, mean value for each experiment, i.e., $\log t_d = 0$. *Drawn curve* is the integral of a normal distribution with SD 0.081 log days. Same distribution was found for several additional experiments in which fewer mice were used (*1*).

Chemical Influences

Photosensitization

Wavelengths shorter than 320 nm, especially those between 280 and 320 nm, constitute the primary carcinogenic action spectrum at least under experimental conditions. These rays also are primarily responsible for acute phototoxic responses to the sun. However, with the introduction of photosensitizing chemicals with suitable action spectra, acute phototoxic responses can be produced by UV-A in amounts that normally do not produce injury (*27*). Repeated UV-A exposures in the presence of photoactive agents such as 8-MOP (*28, 29*), coal tar (*30*), eosin, and hematoporphyrin (*31*) result in a significant cancer yield. Thus a correlation between acute phototoxic injury and eventual cancer formation was apparent. Recently, Forbes et al. (*28*) compared the enhancing effect of two potent photoactive agents, 8-MOP and anthracene; both produce brisk, acute, phototoxic responses with UV-A energy. However, only 8-MOP enhanced photocarcinogenesis when exposed to an artificial solar simulator that contained UV-A and UV-B energy. These findings suggest that qualitative differences occur in acute phototoxic reactions, which determine whether such injuries when repeated will augment or even result in cancer formation. Other possibilities such as photodestructive influences must also be considered.

In recent years, the photoreactions of certain photoactive psoralen compounds with biologic molecules, primarily DNA, have received extensive attention.

In vivo studies with hairless mice demonstrated that 8-MOP- and UV-A-induced phototoxic responses had a profound influence on epidermal, premitotic, semiconservative DNA synthesis but little effect on RNA synthesis (*32*). In addition, early unscheduled DNA synthesis was not observed.

At the molecular level, the mechanism of photoinjury by the psoralens seemed to be associated with the photoaddition of the psoralen compounds to the pyrimidine bases of nucleic acids (*33–35*). A study of the skin photosensitizing effects of a number of furocoumarin compounds with native DNA and RNA indicated a close correlation with photoreactivity with native DNA but not RNA (*35*). More importantly, the photosensitizing psoralens are capable of intercalating between two base pairs of DNA, and this action results in interstrand cross-linkages after irradiation (*36, 37*). Cole and Sinden (*38*) postulated a complicated repair mechanism that requires strand exchanges and repair synthesis, which may be error prone and thus more likely may relate to mutagenesis and carcinogenesis. The difference between the effects of photocarcinogenic psoralen compounds and noncarcinogenic photodynamic agents may relate to their methods of affecting DNA. However, the studies of Grube et al. (*29*) suggested that no correlation exists between cross-links and tumor formation. These mechanisms present important challenges for future examination.

Chemical Carcinogens and Photocarcinogenesis

The polycyclic hydrocarbon carcinogens are potent photosensitizers with action spectra primarily in the long UV range (*39*). Epstein et al. (*40*) and Santamaria and Giordano (*41*) have demonstrated a significant relationship between the photodynamic reactivity and the carcinogenic potential of these compounds. Since the chemicals are photosensitizers, the addition of their inherent carcinogenicity to the cancer-inducing effects of phototoxic insults should accelerate tumor formation. However, when this concept was tested, various conflicting results were noted.

Clark (*42*) in an attempt to clarify the problem used 20-MCA topically followed by varying doses of long UV energy. She noted that exposure to lower energy levels ($4–8 \times 10^7$ ergs/cm^2) shortly after the application of 20-MCA accelerated papilloma formation but did not influence cancer production. When larger amounts of long UV energy were given ($10–18 \times 10^7$ ergs/cm^2), an early acceleration of papilloma formation occurred, but most of these growths regressed. The final results showed a retardation of papilloma and cancer formation. The mechanism of this inhibition has not been clearly established. Morton et al. (*43*) suggested that light energy enhances absorption and leaves less carcinogen in contact with the skin surface. Miller (*44*) and Moodie and co-workers (*45*) proposed that photo-oxidation of the chemical may alter its carcinogenic potential. More recently, the studies of Santamaria and Giordano (*41*) indicated that photo-oxidation of the substrate may limit the carcinogenic efficiency of a compound. Whatever the mechanism, it is clear that the direct influence of UV on the hydrocarbon must be eliminated if their combined carcinogenic effects are to be examined. Using this separation technique, Epstein and Epstein (*46*) demonstrated that subcarcinogenic amounts of UV will stimulate cancer formation initiated by a single application of DMBA. Each animal developed 1 or 2 large tumors, none of which regressed and most were malignant. Squamous cell carcinomas and/or sarcomas were produced (*47*). Multiple papilloma formation and tumor regression did not occur. Therefore, the pattern of tumor development differed from Croton oil promotion of chemically initiated cancer formation. These findings support the concept that tumor promotion by a potent carcinogen results in the progressive formation of malignant tumors (*48*); this seems true whether the promoter is a chemical carcinogen or UV.

Further studies with hairless mice and carcinogenic amounts of UV demonstrated that pretreatment with a single application of DMBA accelerated the rate of tumor appearance and growth and increased the incidence of tumors per mouse (*49*). Almost all the tumors were squamous cell carcinomas; occasional lymph node metastases were also noted.

The pattern of tumor formation resembled that described for summation of carcinogenic responses (*50, 51*). These results suggest that chemical and UV carcinogenic effects are additive in nature. Whether they act at the same sites or through similar mechanisms remains to be determined.

Recently, Stenbäck (*52*) found that pretreatment of mouse skin with UV-B caused a marked increase in DMBA-induced tumors, whereas irradiation after application of the carcinogen significantly decreased tumor induction. These findings further emphasize the variations that occur under experimental conditions and the influence of factors, such as photodestruction of the carcinogen, on the production of tumors.

Chemical Promotion and Photocarcinogenesis

Chemical promotion of UV-initiated cancer formation has been demonstrated in experimental animals. In 1968, we (*18*) reported that repeated applications of Croton oil after a single UV exposure did result in cancer production. Subsequently, Pound confirmed these results in his animal system (*19*). Whether the Croton oil applications in these studies served to accelerate tumor growth that started with the single UV exposure or functioned as a promoter by transforming UV-initiated cells into a tumor-forming population has not been determined. However, in contrast to the effects of initiation with a polycyclic hydrocarbon carcinogen, only one growth was produced per tumor-bearing mouse. Multiple papilloma formation and significant tumor regression typical of chemical initiation with Croton oil promotion was not noted. Thus these findings indicate that not only does the process of cancer formation begin with the initial UV exposure but also the characteristics of the tumor appear to be established by the light energy prior to the chemical stimulation.

Of interest is the study by Stenbäck (*53*) in which he used a different animal and irradiation system, which confirmed the tumor-promoting effects of Croton oil following a single

TABLE 1.—*Retinoic acid promotion study*[a]

Weeks after starting UV	Tumor formation[b]					
	Tumors >4 mm^3			Tumors >50 mm^3		
	Group I	Group II	*P*	Group I	Group II	*P*
29	7/25	2/30	0.083	5/25	1/30	NS
30	7/25	2/30	0.083	6/25	1/30	0.06
31	8/25	2/30	0.04	8/25	1/30	0.013
32	8/25	2/30	0.04	8/25	1/30	0.013
33	9/25	4/30	0.015	8/25	1/30	0.013
34	10/25	8/30	NS	8/25	3/30	0.094
35	12/25	11/30	NS	8/25	3/30	0.094

[a] Group I received retinoic acid and UV, and group II received placebos and UV.

[b] Values indicate number of mice of total treated that developed tumors. NS = not significant.

UV exposure. However, the mice developed only a few benign tumors, some of which regressed.

These studies indicate that chemical promotion of UV carcinogenesis can occur under experimental conditions. However, Croton oil is not likely to function in this capacity in humans. We examined tumor promotion with two chemicals that might relate to clinical problems: retinoic acid, which is extensively used in the treatment of acne and BCNU, a nitrosourea compound being used topically in the treatment of mycosis fungoides and experimentally for psoriasis.

A 0.3% retinoic acid cream and the cream base were used for topical applications (obtained from Hoffmann-La Roche Inc., Nutley, N. J.). The animals were divided into 2 groups. The right flanks of the 38 mice in group I were exposed to 1.38×10^3 mJ/cm^2 of the UV energy three times a week for 10 months. The retinoic acid cream (approximately 0.5 g) was applied with a wooden applicator to the right and left flanks of each mouse immediately after UV exposure for the first 4.5 months. The 31 mice in group II received UV exposures as in group I, but the cream base was used in place of the retinoic acid applications for the first 4.5 months.

Within the first 3–4 weeks, all the group I mice developed erythema and desquamation that continued throughout the study but subsided to some extent after the retinoic acid applications were discontinued. Thirteen mice died by 4.5 months; this necessitated cessation of the topical applications on the remaining animals. The cream base caused no observable cutaneous or systemic changes in the animals.

No tumors occurred on the unirradiated left flank skin in any of the mice, which indicated that neither the cream base nor the retinoic acid was carcinogenic under the circumstances of the study.

The onset of tumors greater than 4 mm^3 occurred by about 6 months in both groups. However, they appeared more rapidly in group I until week 34, at which time approximately equal numbers of mice were involved in both groups (table 1).

Until week 35, tumors greater than 50 mm^3 appeared earlier and were more prevalent in mice treated with retinoic acid. Thereafter, no significant difference was noted in comparative tumor incidence between the placebo- and retinoic acid-treated animals. The tumors in both groups were invasive growths which became enlarged and eventually were greater than 100 mm^3. Of the tumor-bearing mice in groups I and II, 85 and 86%, respectively, had tumors greater than 100 mm^3. From these results and under our study protocol, we concluded that the topical applications of retinoic acid promoted UV-induced cancer formation.

Since we used an extremely high and irritating concentration of retinoic acid and our studies were done on experimental animals, it is reasonable to question the relationship of these findings to human use. Though we cannot avoid the animal model aspect of such investigations, we are now examining much lower, nonirritating concentrations of the chemical.

BCNU was dissolved in acetone in a concentration of 5 mg/ml; the solution was freshly prepared before each application. The hairless mice were divided into 4 groups. The 40 mice in group I and the 36 in group II received one application of BCNU (0.1 ml = 0.5 mg) once a week and UV-B exposures (1.25×10^3 mJ/cm^2) three times a week for 52 weeks; both treatments were applied to the posterior half of the back. The 29 animals in group III and the 40 in group IV were given only BCNU and acetone applications, respectively.

Although tumors failed to develop in the animals that received no UV, the first tumors occurred at about the same time in groups I and II and appeared more rapidly and grew faster in group I as compared with group II (table 2). By the 11th month, most of the mice in both groups had large tumors.

These results indicate that BCNU promoted UV-induced cancer formation under the circumstances of this study. Again, findings in experimental animals cannot be translated to human responses. However, the avoidance of extensive sun exposure or UV therapy while BCNU is being applied topically would seem to be a reasonable precaution.

QUALITATIVE STUDIES

Squamous Cell Carcinomas

The sarcomas produced on the ears of albino mice were satisfactory for the quantitative studies described previously. However, the primary tumors produced in human skin are epithelial in nature. The hairless mouse proved to be an excellent model for studying qualitative changes, since squamous cell carcinomas can be produced with UV energy almost to the exclusion of sarcomas in this animal.

TABLE 2.—*BCNU tumor promotion study* [a]

Time in months after starting UV	Tumor formation					
	Tumors >4 mm^3			Tumors >50 mm^3		
	Group I	Group II	*P*	Group I	Group II	*P*
3	3/40	2/34	NS	1/40	1/34	NS
4	9/38	2/34	0.0361	1/38	1/34	NS
5	13/34	1/34	0.0003	5/34	1/34	0.0872
6	15/33	3/34	0.0007	6/28	3/34	0.1609
7	22/33	4/34	0.0000	9/28	3/33	0.0240
8	26/33	10/33	0.0001	14/25	6/33	0.0027
9	29/30	18/33	0.0001	20/22	13/33	0.0001
10	29/30	24/33	0.0094	21/22	20/33	0.0037

[a] In addition to UV light, groups I and II received BCNU and acetone, respectively.

Using this model, we evaluated the progressive development from benign hyperplasia through stages of actinic keratosis-like lesions to frank invasive malignancy (figs. 1–4). Histochemical (*54*), immunofluorescent (*55*), and the radioactive tracer $[^3H]$dThd (*56*) techniques were used to evaluate anatomic changes and cellular kinetics. These studies demonstrated an accumulation of acid mucopolysaccharides and a loss of mature collagen in the upper dermis and the proliferation of mast cells and fibrocytes. The epidermal–dermal BM as demonstrated by mucopolysaccharide stains and light microscopy showed a progressive thickening with the development of benign epidermal hyperplasia. As irregular and abnormal epidermal proliferation occurred, the BM became thicker, irregular, clumped, and frayed in appearance and, with frank invasive malignancy, it disappeared altogether. In contrast, the antigenic BM as demonstrated by indirect immunofluorescence studies with pemphigoid serum remained unchanged until invasion occurred (*55*). Thus this antigenic BM appears different from the PAS staining structure.

However, both were lost when invasion occurred. Similar breaks have been noted with invasion of human skin cancers (*57*) and chemically induced experimental tumors (*58*). Electron microscopic studies have suggested that microprojection of cells through the basal lamina may represent the earliest stage of tumor invasion (*59*).

In our epidermal cell kinetic studies (*56*), we observed a progressive increase in the number of germinative basal cells synthesizing DNA and dividing, with reduction of the DNA synthesis and G_2-periods associated with the development of epidermal changes. In addition, a progressive reduction of the cell transit time through the epidermis occurred despite its increasing thickness. With frank malignancy, the presence of a germinative cell layer was lost and mitoses, many abnormal in appearance, were present throughout the tumor. Thus the process of cancerization was characterized by acceleration of cell formation, maturation, and turnover. Furthermore, the epidermal germinative basal cell appeared to be the primary or initial site of abnormal proliferation.

Although these epidermal and BM findings are striking, UV-induced changes in the dermis indubitably are part of the carcinogenic process. Actinic elastosis has been considered important in the development of cutaneous cancers because of its frequent association with skin cancer formation. However, Sams and his co-workers (*60*) and Graham and Helwig (*61*) demonstrated that actinic elastosis is not essential for the development of epidermal cancers. In addition, the experimental production of elastosis in animals has not been associated with cancer formation nor have UV-induced experimental cancers been dependent on the presence of this change (*8, 47*).

Whereas the association of actinic elastosis and skin cancer formation appears to be fortuitous, other less obvious changes may well influence tumor development. Connective tissue changes have been correlated with the development of basal cell epitheliomas in man and in experimental animals. These include normal collagen and elastic tissue dissolution, proliferation of argyrophilic fibers (most likely young collagen), increased amounts of acid mucopolysaccharides, increased numbers of mast cells and fibroblasts, deposition of PAS-positive material around the advancing epithelium and, at times, the formation of new elastic tissue (*58, 62–64*). Striking changes in the dermal vasculature have also been noted in experimentally induced as well as naturally occurring epidermal neoplasms (*61, 65*). These may be partially related to a tumor angiogenic factor as described by Folkman et al. (*66*). Such a factor or factors have been reported to be associated with human skin tumors (*67, 68*). Thus a number of dermal changes do occur that may be partly responsible for epidermal cancer formation.

Malignant Melanoma

Epidemiologic studies suggest that melanoma formation in certain anatomic locations may be induced or aggravated by sunlight energy. However, the role of light in the induction of these growths has not been conclusively demonstrated.

Benign melanocytic tumors have been induced in experimental animals, primarily hamsters and pigmented mice, with chemical carcinogens.

Attempts to produce malignant transformation with various traumatic procedures were unsuccessful (*69*). However, Epstein et al. (*70*) did induce this transformation in hairless mice with UV energy shorter than 320 nm. Repeated exposures of benign blue nevi (fig. 5) for several months resulted in the production of large invasive

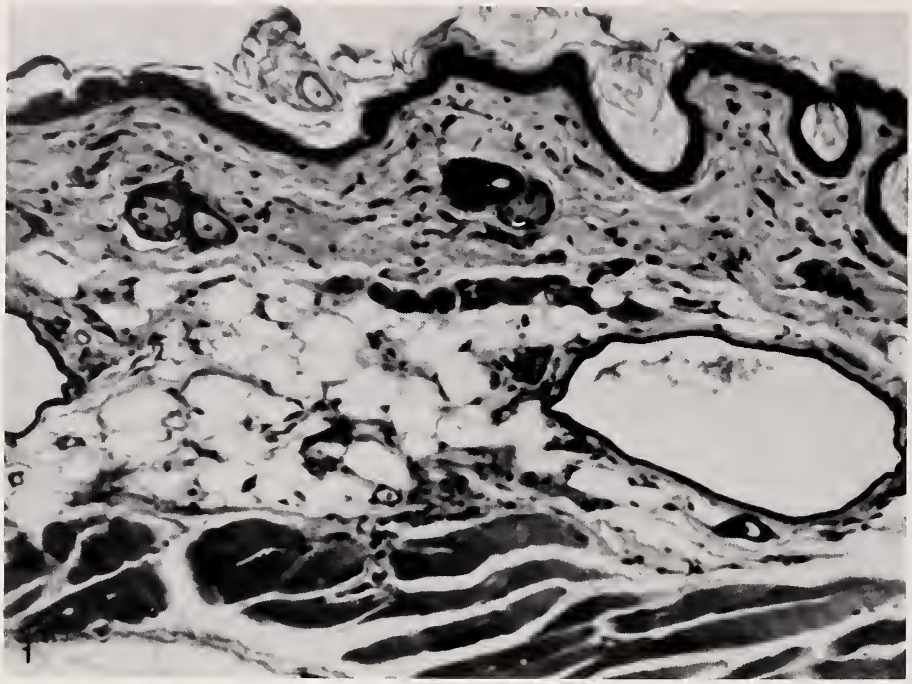


FIGURE 1.—Normal hairless mouse skin. H & E. × 75

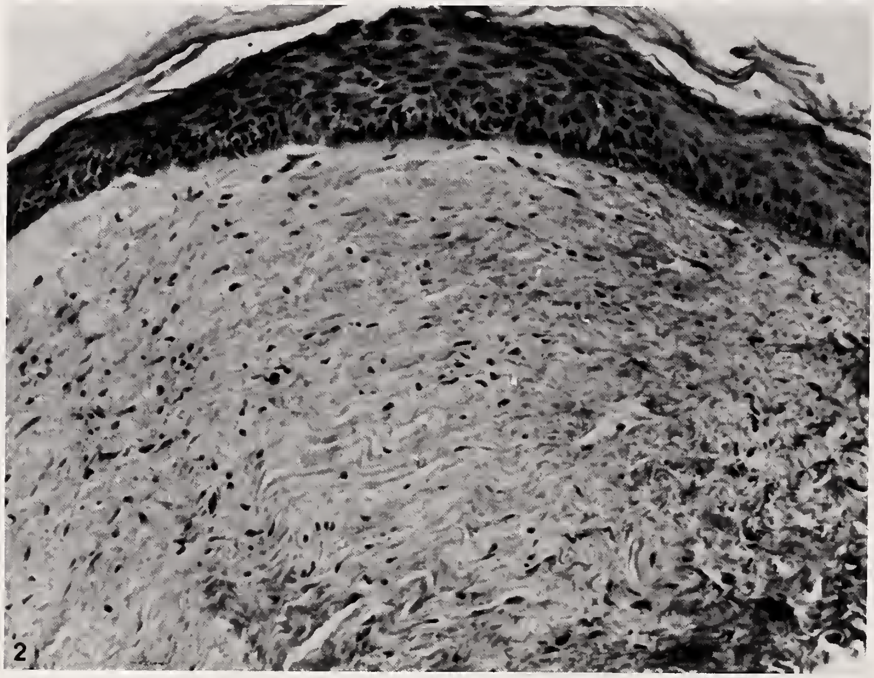


FIGURE 2.—Hairless mouse skin. Benign epidermal hyperplasia and thickening of the dermis after 8 wk of UV irradiation. H & E. × 75

melanocytic tumors (fig. 6) with histologic (fig. 7), autoradiographic, and electron microscopic characteristics of malignancy. In addition, several of these tumors metastasized to the regional lymph nodes. These results indicate that benign pigmented lesions can be transformed to malignant growths with UV energy in experimental animals. Whether a similar process occurs in human skin remains to be established.

IMMUNE RESPONSES AND PHOTOCARCINOGENESIS

Tumor-specific antigens have been demonstrated in chemically induced cutaneous cancers. Kripke (*71*) noted that UV-induced cancers in mice were even more antigenic than these chemically induced tumors. This antigenicity can conceiva-

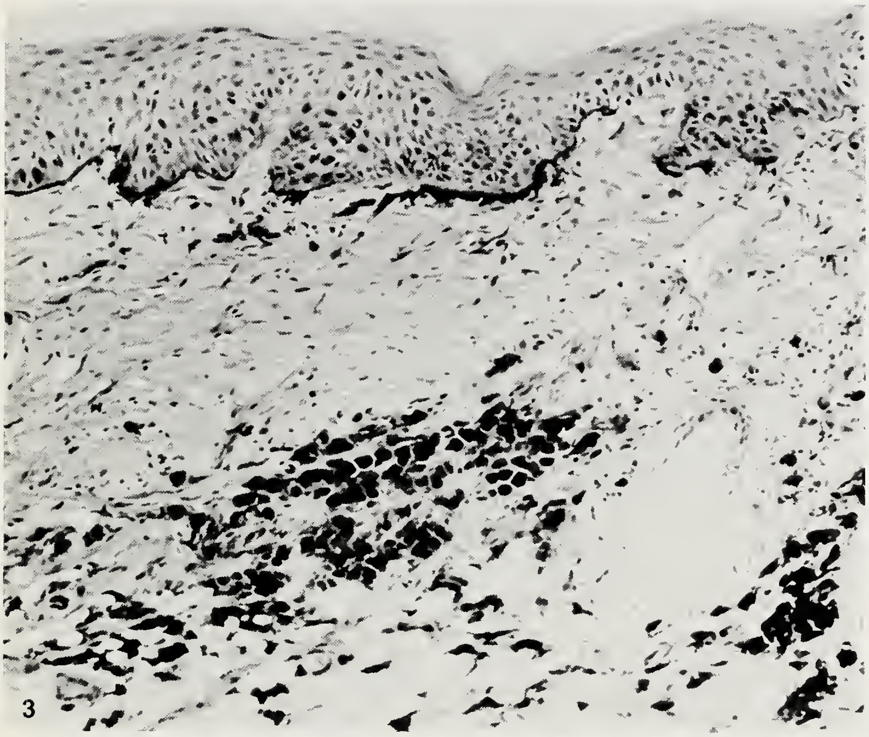

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FIGURE 3.—Hairless mouse skin in premalignant stage after 32 wk of UV irradiation showing irregular hyperplasia of the epidermis and a thick irregular brush-like epidermal–dermal BM. Mowry colloidal iron. × 75

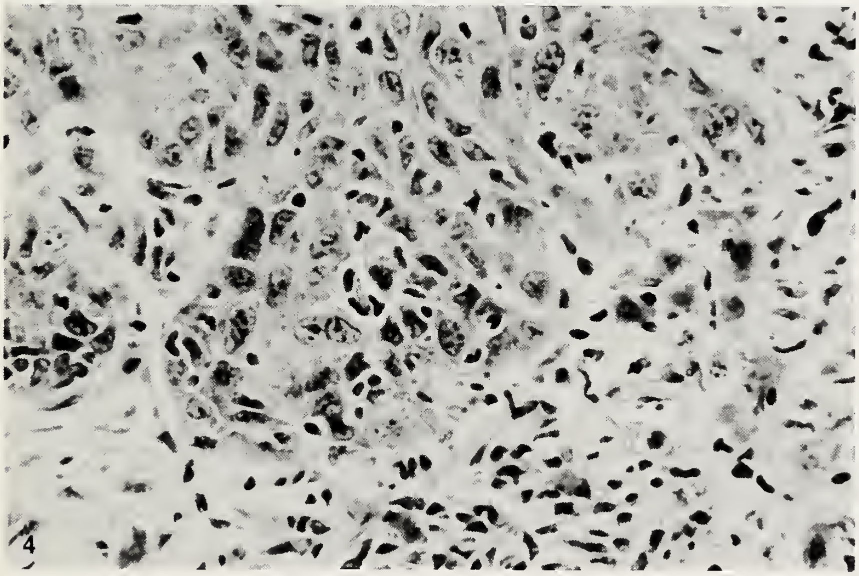
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FIGURE 4.—Hairless mouse skin. Frank, invasive squamous cell carcinoma. Abnormal mitoses, nuclear atypicality and loss of cellular polarity. H & E. × 125

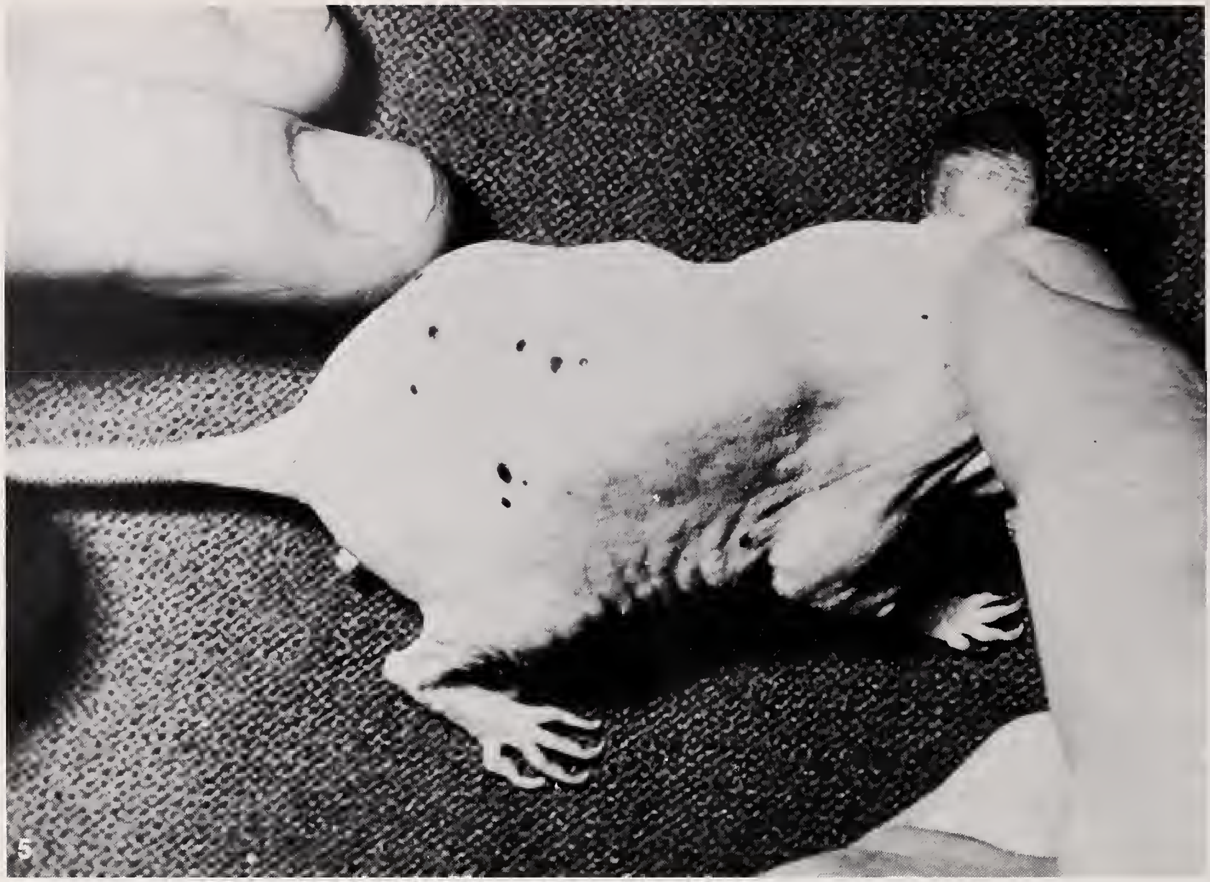

FIGURE 5.—Hairless mouse with benign blue nevi produced by a single application of DMBA (*70*).

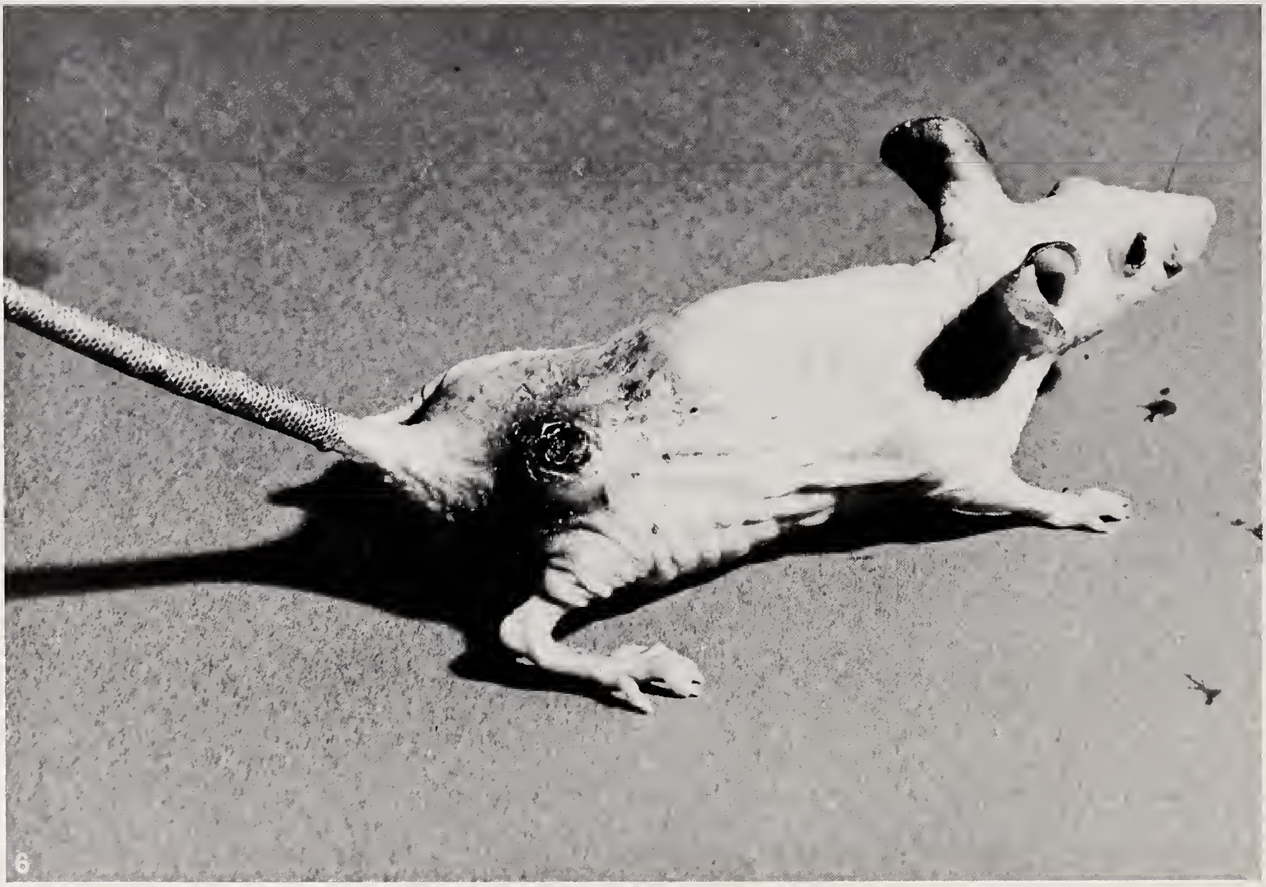

FIGURE 6.—Large melanoma resulting from UV stimulation of a benign blue nevus (*70*).

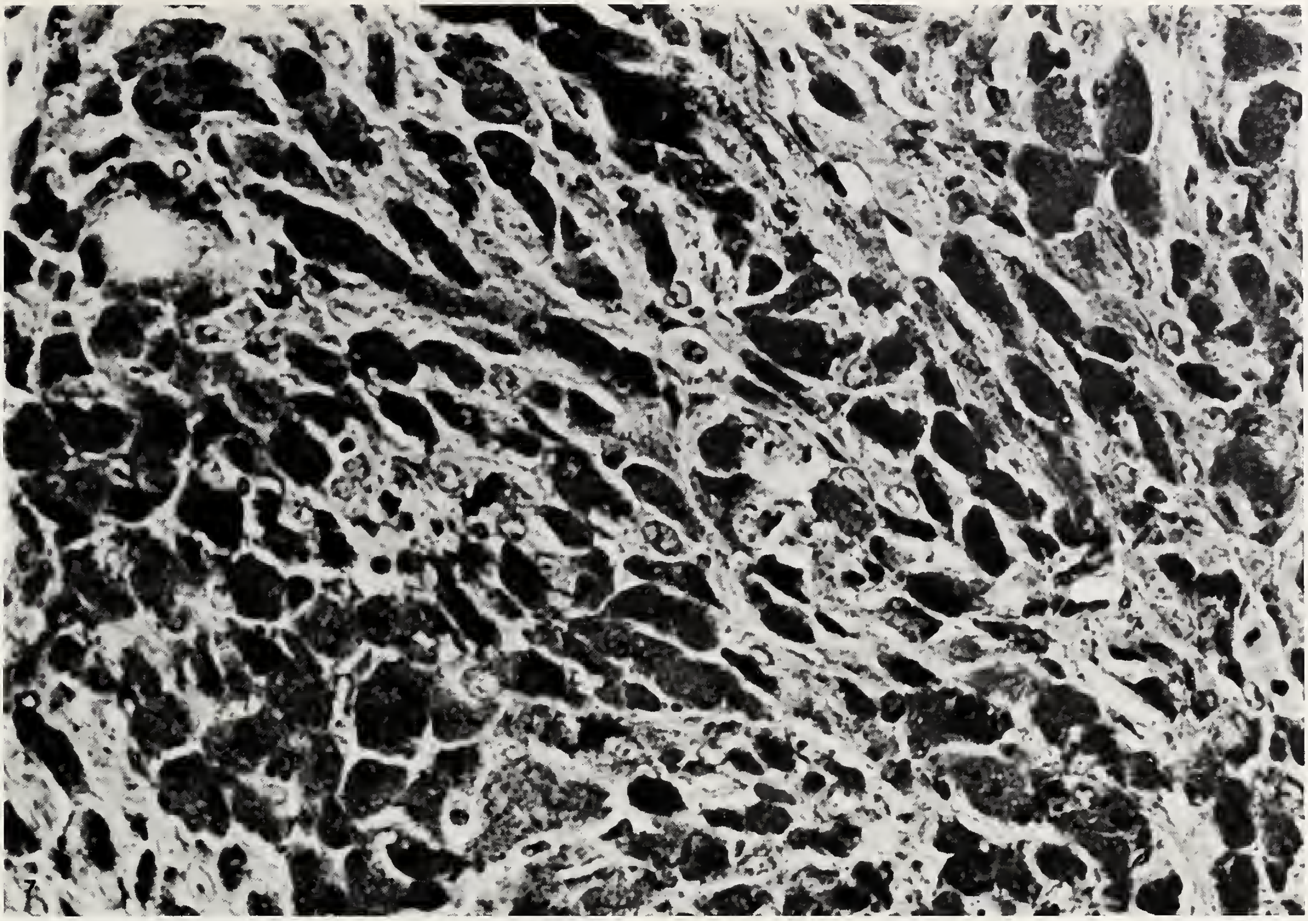

FIGURE 7.—Nuclei of the malignant melanoma cells were abnormal in size, shape, and chromatin pattern (*70*). H & E. × 100

bly be used to detect the presence of such tumors when the methodology is refined.

In contrast, antigen deletion or loss of tissue specificity also occurs during the development of cutaneous cancers (*72*). De Moragas et al. (*73, 74*) used intercellular antibody-containing pemphigus sera, BM antibody-containing pemphigoid sera, and indirect fluorescence antibody techniques to demonstrate a loss of epidermal antigens associated with human skin cancers. In squamous cell carcinomas, they noted that the loss of intercellular and BM antigenic material varied inversely with the degree of cellular anaplasia. Subsequently, Muller and Sutherland (*75*) examined the presence of intercellular and BM antigenic material, using the same type of serum antibodies (pemphigus and pemphigoid) in the progressive changes occurring during the production of cutaneous squamous cell cancers with UV and a chemical carcinogen. Intercellular antigens were not lost until the epidermal cells reached a premalignant stage of abnormality. As in the histochemical findings, the BM antigenic response became diminished or was lost with tumor invasion. Jekot et al. (*55*) confirmed the loss of the antigenic BM response with frank malignant invasion of UV-induced experimental tumors.

In addition, Hersch et al. (*76*), using antisera to a normal rat and mouse epidermal fibrous protein, noted a loss of antigenic reactivity to this antigen with the progressive development of UV-induced cancer formation. Whether this was due to antigen deletion or masking of the antigenic sites remains to be determined. At present, the latter concept seems more plausible.

Recently, the importance of immune defenses and cancer control has received a significant amount of attention. This concept has gained support from the association of cancer with a number of immune deficiency states and autoimmune diseases in man and animals. The importance of immune competence in controlling cancer has become particularly important in the realm of human cancer formation with the expansion of organ transplantation and the need for suppression of the recipient's immune response to prevent rejection.

Lymphomas have been the most common cancers associated with organ transplants. Some evidence suggested that this type is related to the presence of foreign tissue as well as immunosuppression (*77*). However, incidence is also increased in other tumors, including cutaneous keratoacanthomas and squamous cell carcinomas (*78–80*). These cutaneous lesions depend on actinic damage and the anticancer agents but do not seem to require foreign protein. Whether these tumor-enhancing effects in animals and man are related to immunosuppression or they directly affect neoplasia induction remains to be determined.

Additional evidence for the importance of immunologic surveillance was presented by Nathanson et al. (*81*). Immunosuppression with rabbit antimouse lymphocytic serum of hairless mice during UV-induced photocarcinogenesis resulted in much earlier appearance of tumors than in the saline-treated control animals. Suppression of lymphocyte-mediated, delayed immunity clearly reduced the appearance time as well as increased the incidence of UV-induced tumors.

In contrast to the findings with high-dose administration of small amounts of drugs, such as MTX, for prolonged periods for psoriasis apparently is not generally responsible for increased skin cancer formation in humans (*82*), though observations to the contrary have been reported (*83, 84*).

Recently, we examined the effects of low-dose administration of MTX and hydroxyurea on UV carcinogenesis in hairless mice. These antimitotic agents are commonly used for long periods in the treatment of psoriasis in humans.

The hairless mice were divided into 3 groups. MTX was dissolved in NSS at a concentration of 0.1 mg/ml. Three times per week, group I (42 mice) received 0.1 ml ip after UV irradiation of 6.57×10^2 mJ/cm^2 to the posterior half of the back for 12 months. Group II (45 mice) received 0.1 ml NSS following the UV exposures as in group I, and the 36 mice in group III were given only the MTX ip. Only 1 tumor, which appeared at 9 months, occurred in group III. Most of the tumors in groups I and II were noted between 8 and 12 months, and no significant difference in tumor incidence or growth occurred between these groups. By 12 months, most of the animals in these 2 groups had tumors greater than 100 mm^3.

These results indicate that low doses of MTX administered over a long period did not potentiate UV-induced carcinogenesis.

Hydroxyurea was dissolved in NSS (freshly prepared each week) at a concentration of 75.6 mg/12 ml. The hairless mice were divided into 2 groups. Group I (40 mice) received 0.1 ml (0.63 mg) of the hydroxyurea ip 5 and 2 days before and then three times a week following UV irradiation for 52 weeks. The mice were exposed to 11.75×10^2 mJ/cm^2 of UV energy shorter than 320 nm three times a week for the duration of the study. Group II (41 mice) received NSS ip before and after UV irradiation as did group I.

The onset of the first tumors greater than 4 and 50 mm^3 appeared 3 weeks earlier in group II (NSS + UV) compared with group I (hydroxyurea + UV). In general, the tumors appeared more rapidly and grew faster in group II. This difference was most notable between weeks 36 and 44.

From these results, we concluded that long-term, low-dose hydroxyurea did not potentiate UV-induced cancer formation but actually reduced it under the conditions of this study. Long-term administration of 6-mercaptopurine (*81*) and prednisone (*85*) will also reduce UV-induced tumor formation in experimental animals. In contrast, azathiaprine in long-term toxic doses accelerated UV carcinogenesis (*85*).

Thus the experimental findings support the concept that at least 4 of these medications may not accentuate sunlight-induced cancer formation when they are used in the treatment of benign dermatoses in humans. The discrepancy with azathiaprine may relate to the comparatively high dose used.

Perhaps the most exciting discoveries relating to immune responses and UV carcinogenesis developed from the studies of Kripke and Fisher (*86–88*). Using a variety of techniques, they demonstrated that chronically UV-irradiated mice do not reject highly antigenic UV-induced cancers rejected by unirradiated syngeneic recipients. This susceptibility of the primary hosts even occurred with antigenically dissimilar UV-induced neoplasms and appeared long before the UV-induced tumors did. Their findings indicated that UV irradiation specifically altered the immunologic responses of these animals to UV-induced cancers (*89*).

DNA DAMAGE AND PHOTOCARCINOGENESIS

As noted earlier, many aspects of UV-induced cancer formation have been examined, measured, counted, and reviewed. However, the molecular pathogenic mechanisms involved in this process remain to be determined.

Some have suggested that UV may induce mitosis-stimulating and/or reduce mitosis-inhibiting factors that result in cell proliferation. Loss of immune surveillance and control may be important. UV production of the potential chemical carcinogen cholesterol-α-oxide in the skin has been reported (*90*). Perhaps the most intriguing avenues for investigation at this time relate to UV effects on cellular DNA.

Direct inhibition of DNA synthesis represents one of the earliest events after UV irradiation in the human and murine epidermis in vivo (*91, 92*) and in cultured mammalian cells (*93*). Though a number of potential injuries could occur, pyrimidine dimer formation (primarily between thymine bases) and repair of this injury have received an extraordinary amount of attention within the last decade. Currently, three repair mechanisms for DNA base damage in bacteria have been described: photoreactivation, excision repair, and postreplication repair (*94*). All three systems have been noted in various mammalian cells, including human cells (*95–100*), though the dark excision repair process has received the greatest emphasis to date. Evidence has been reported that this repair mechanism occurs in human epidermis in vivo (*91*) and in cultured cells (*95*).

The discovery in humans that the photosensitive disease XP is characterized by a defect in this dark repair enzyme system (*101*) has supplied a model for its evaluation in human skin. Bacteria with a defect in excision repair not only are more sensitive to the killing effects of UV radiation but also form more mutations after irradiation. The primary photosensitive problem in patients with XP is their inordinate sensitivity to the carcinogenic effects of sunlight.

Since Cleaver (*101*) first described this defect in cultured XP fibroblasts, repair processes in this disease have been examined extensively. The defect has been described in the epidermis in vivo (*102*), and in lymphocytes of these patients (*103*). Setlow et al. (*104*) obtained evidence that the primary site of the enzymatic defect occurs in the initial incision stage. However, perhaps the most exciting findings have developed from complementation studies. Using this technique, several investigators found a number of distinct genetic defects in this enzyme system in cells from various patients with the clinical picture of XP (*105–107*). The studies of Sutherland et al. also suggest that photoreactivation repair may be defective in some of these patients (*100*). Recently, Lehman et al. (*97*) reported that postreplication repair is defective in the XP variant in which the excision repair process is normal.

Setlow and Hart (*108*) demonstrated that photoreactivation procedures will reduce UV-induced tumor formation in a fish model; therefore, a defect in photoreactivation can lead to tumor production in appropriate experimental systems. In contrast, Zajdela and Latarjet (*109*) demonstrated that applications of caffeine, a potent inhibitor of postreplication repair, resulted in fewer UV-induced skin cancers in animals. This finding suggests that error-prone repair is more important than defects that will lead to cell death. Thus the disease or diseases included under the name "xeroderma pigmentosum" has supplied a remarkable model for examining DNA repair mechanisms in human cells. How any of the defects noted relate to cancer formation in XP, much less the general field of UV carcinogenesis, requires further study.

SUMMARY AND CONCLUSIONS

The etiologic role of sunlight in the development of human skin cancer was suggested initially by a number of astute observations reported around the turn of the 20th century. Subsequently, epidemiologic studies, including the association of sun exposure with the distribution of lesions, the pigmentation and genetic make-up of different populations, and the amount of hours of sunlight available geographically, have supplied a large body of circumstantial evidence supporting the relationship between sun exposure and development of human skin cancer (*1–5*).

The evidence is circumstantial because no one has actually induced skin cancer in people with light energy under controlled conditions. However, the epidemiologic evidence strongly supports the influence of sun exposure on the development of basal and squamous cell cancers. In addition, at least certain types of melanomas appear to be initiated and/or aggravated by sunlight.

Experimental animal and tissue culture studies have added a vast catalog of information to the clinical observations, e.g., quantitative and qualitative cancer development data; the influence of chemical carcinogens, chemical promoters, and photosensitizers on UV carcinogenesis; characterization of the antigenicity; the occurrence of antigen deletion; immunologic control as it relates to UV-induced cancers; and the role of DNA damage and repair in the development of these tumors.

Despite these extensive clinical and experimental studies, the role of sunlight in and the mechanisms responsible for cutaneous cancer formation remain to be determined. There are significant deficiencies in our knowledge of this process. Among the myriad of potential aspects in need of study, I consider the following three parameters of utmost importance: 1) expansion of the epidemiologic information to include a complete profile of cancer incidences as they relate to latitude, genetics, and potential UV-B exposure; 2) extension of our knowledge of immune mechanisms as they relate to diagnosis and control, and 3) further evaluation of molecular events as they relate to the development of these tumors.

Obviously, much work must be done before the complexities of UV carcinogenesis will be sorted out and delineated.

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Action Spectrum for Ultraviolet Carcinogenesis[1]

Robert G. Freeman[2]

ABSTRACT—Previous observations and experiments have shown that the potent carcinogenic wavelengths for skin cancer in man are found in the 290- to 320-nm range, although shorter and longer wavelengths can also have an effect. The short wavelength limit (290 nm) is determined by the solar emission reaching the earth's surface, a parameter that varies greatly with season, time, and atmospheric conditions. The long wavelength limit (320 nm) was based on observations of the effect of window glass filtration and on comparison of the effect of mid and long UV radiation. It provided little information as to the efficacy of wavelengths in this range. We performed a series of experiments to provide more specific comparative data by testing the hypothesis that the action spectrum for carcinogenesis paralleled that for erythema. Albino mice were exposed to the emission of a diffraction grating monochromator (5 nm half power bandwidth) at 290, 300, 310, and 320 nm. Energy levels were proportional to values for the erythema spectrum of untanned Caucasian human skin. Exposures were given thrice weekly until 50% of the mice had developed tumors. Squamous cell carcinomas developed at approximately the same rate and frequency when the UV exposure was proportional to that for erythema, which indicated a decreasing potency from 300 to 310 to 320 nm. No tumors occurred in mice exposed to 290 nm.—Natl Cancer Inst Monogr 50: 27–29, 1978.

ACTION SPECTRUM FOR UV CARCINOGENESIS

The action spectrum for UV carcinogenesis has not been delineated precisely. The technical and ethical problems inherent in this project are immense and are prohibitive in man. Determination of a spectrum with an animal model is also difficult because of the numbers of animals required to establish significance, the small field size of emission from high intensity monochromatic light sources, and the long induction time for tumor development and growth. In the short-wavelength UV range (below 250 nm), potent light sources have not been available for this type of study. However, data in this range would not be applicable to carcinogenesis in man on the earth's surface, since such wavelengths (below 290 nm) do not reach the surface of the earth in significant quantity.

Although no action spectrum has been delineated, scientists are aware of two areas pertinent to the spectrum for carcinogenesis in man. The first is physical and meteorologic in nature and deals with the short-wavelength end of the solar spectrum, i.e., the energy available at the earth's surface that can induce cancers. The second is photobiologic and consists of data obtained from animal experimentation.

THE SHORT UV END OF THE ACTION SPECTRUM

Experiments with laboratory animals have demonstrated tumor development following exposure to wavelengths of 250 and 254 nm. Professor Ian Magnus (personal communication) has observed that 1) tumors developed after mice were exposed to monochromatic UV of 250-nm wavelength, and 2) the germicidal lamp emitting primarily at 254 nm is an effective tumorigenic light source (*1*). Ionizing radiation is carcinogenic, but little is known of intervening UV range shorter than 250 nm.

From a practical standpoint, this information is not relevant to sunlight-induced cancer in man because these shorter wavelengths do not reach the earth's surface in significant amounts. More pertinent to the problem of human skin cancer is the sun's spectrum at the earth's surface and the effects on it of atmospheric conditions, pollution, ozone and ozone depletion, altitude, and other parameters.

The wavelength minimum reaching the earth's surface lies in the mid-UV range and varies with the zenith angle. Thus it is constantly changing and fluctuating with the seasons, time of day, and with atmospheric conditions; the intensity of mid-UV wavelengths varies similarly. These factors have been presented and reviewed in detail (*2*).

Generally, the shortest wavelengths reaching the earth's surface are at approximately 290 nm. This wavelength minimum of 290 nm may be true for certain geographic areas, but we recorded minimum wavelengths at approximately 30° N latitude and with a zenith angle as low as 7° at 294 nm and often longer, i.e., between 296 and 300 nm. Extensive data regarding the solar spectrum are available, e.g., those reported by Bener (*3*). Further discussion of this aspect of carcinogenesis will not be included here.

THE LONG WAVELENGTH END OF THE SPECTRUM

Roffo (*4*) contributed much to our present knowledge about wavelength dependency when he found skin tumors in mice exposed to natural sunlight or to mercury arc lamps. This action, blocked entirely by an ordinary window glass filter, indicated that the major carcinogenic potency is with wavelengths shorter than 320 nm. Long UV light sources also are ineffective.

Experimental studies during the last 30 years revealed that skin cancers can be produced in laboratory animals (*4–7*), notably mice, by exposure to 1) mercury arc lamps, 2) sun-

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Abbreviation: TD50 = median toxic dose.

[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] Department of Pathology and Division of Dermatology, University of Texas Health Sciences Center, Southwestern Medical School, Dallas, Texas 75235.

light, 3) germicidal lamps, 4) ionizing radiations, and 5) monochromatic light of 254-nm wavelengths. Cancers do not occur in these animals if the 1) radiation is filtered through window glass, 2) light source is long UV, or 3) light source is visible light alone. These experiments with various artificial sources have been repeated and confirmed many times but have added little to our knowledge of the specific wavelengths that are effective or of their capacity to induce cancer. The longest wavelengths generally considered to comprise the spectrum are approximately 320 nm, i.e., the shorter wavelength limit of window glass transmission. Window glass, which has a sharp cut-off in transmission below 320 nm (*8*), filters all wavelengths well in the mid-UV range and reduces the quantity of potent carcinogenic UV energy to an ineffective level. This result is due to a reduction in total dose, irrespective of wavelengths within the mid-UV range. It does not provide information regarding the relative potencies for carcinogenesis of the various wavelengths shorter than 320 nm nor for the effectiveness, even if minimal, of wavelengths longer than 320 nm. Recent experiments dealt with these two questions pertinent to the longer wavelength end of the carcinogenic action spectrum.

Relative Effectiveness of Monochromatic Mid-UV

We compared high-intensity monochromatic UV wavelengths of 290 to 320 nm for their efficacy in inducing tumors in mice by testing the hypothesis that carcinogenic effectiveness was proportional to erythemal activity (*9*).

In this study, groups of albino mice were exposed to the emission from a high intensity diffraction grating monochromator with a half power bandwidth of 5 nm (*10*). Irradiation was given thrice weekly on one ear of each mouse; the opposite ear served as a control. The end point was the time required for 50% of the surviving animals to develop tumors (TD50).

When mice were irradiated with the same dose of UV energy at 300 and at 310 nm, it was clear that UV radiation of a 300-nm wavelength is more likely to produce skin tumors than is one of 310 nm. No tumors occurred at 310 nm nor was any visible damage observed. The first tumor appeared in the 300-nm group after 323 days, and 50% of the animals developed tumors by 458 days (table 1); all were squamous cell carcinomas.

To test the hypothesis that carcinogenic effectiveness of UV is proportional to that of the erythemal potency of UV, we performed a second series of experiments in which albino mice were exposed to monochromatic UV of 290, 310, and 320 nm wavelengths in dosages proportional to the threshold dose for erythema production in untanned human skin (*11*).

Although no tumors appeared in mice exposed to low levels (60 mJ/cm^2/wk) at 310 nm, tumors did appear when UV energy of 310 nm was given to a group of mice at a dose of 750 mJ/cm^2/week. The first appearance of tumors and the TD50 closely paralleled those receiving 60 mJ/cm^2/wk at 300 nm (table 1). Of the 8 tumors found in 16 survivors, 5 were squamous cell carcinomas, 2 were fibrosarcomas, and 1 was an angiosarcoma. Tumors also appeared in 2 of 5 mice receiving UV energy of 320-nm wavelengths after 417 and 464 days. No tumors nor visible damage developed in mice receiving 290 nm UV.

The occurrence of tumors in mice exposed to monochromatic UV at 300, 310, and 320 nm in quantities proportional to erythemal effectiveness supports the hypothesis stated above.

Carcinogenesis by Long UV

It has been assumed that long UV energy (320–400 nm) had little or no carcinogenic activity except in the abnormal presence of a photosensitizing chemical, such as psoralen. This assumption is based primarily on the early studies in which the mid-UV (280–320 nm) was removed by window glass filtration, which resulted in elimination of the carcinogenic effect of the light source. Thus it is assumed that the carcinogenic potency is due to mid-UV and that long UV was ineffective. The latter does not necessarily follow, since the filtration may merely have reduced the total energy to subthreshold levels. The potency of mid-UV has been established repeatedly in experiments in which selective filtration, line spectra, and monochromatic sources were used.

Recently, Forbes (*12, 13*) reported results of an experiment that indicated that long UV may indeed be carcinogenic at a level conceivably important in human carcinogenesis. He observed carcinomas after 20 weeks of continuous exposure of hairless mice to a flux of about 1,000 μW/cm^2 from a band of F-40 T12 fluorescent black-light lamps with the mid-UV filtered. The conditions of their study were not comparable to the human situation that would involve continuous, intense exposures with no opportunity for photorecovery to occur. However, the demonstration that long UV can be carcinogenic at any level is significant, especially considering the large amounts of long UV in sunlight.

The large quantity of long UV in sunlight, the possibility of even a weak carcinogenic effect, and the possibility of interaction or additive reactions between long and mid-UV must be considered in any future experiments.

TABLE 1.—*Carcinogenesis with monochromatic light*

Wavelength, nm	Weekly dose, mJ/cm^2	Energy levels, mJ/cm^2	TD 50		Ratio of total dose/MED[a]
			Days	Total dose, mJ/cm^2	
290	42	14			
300	60	20	458	3,400	243
310	60	20	—	—	—
310	750	250	465	45,500	250
320	4,950	1,650	(417, 464)		

[a] MED = minimum erythema dose.

SUMMARY

The UV content of sunlight is the major cutaneous carcinogen in man. The most potent wavelengths are found in the mid-UV range (approximately 290–320 nm), and their carcinogenic effectiveness is proportional to that for erythema induction. For shorter wavelengths, the solar UV reaching the earth's surface varies greatly with atmospheric conditions, and this wavelength minimum determines the effective shorter wavelengths available for induction of cancer in man.

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Experimental Ultraviolet Photocarcinogenesis: Wavelength Interactions and Time–Dose Relationships[1, 2]

P. Donald Forbes, Ronald E. Davies, *and* Frederick Urbach[3]

ABSTRACT—Tumors were induced in the skin of SKH hairless mice by exposure to fluorescent FS sun lamps or to a long-arc xenon solar simulator. Tumors developed about equally well with varying amounts of UV-A radiation ($\lambda > 320$ nm) given simultaneously. In contrast, incremental changes in the UV-B region ($\lambda < 320$ nm) led to substantial increases in carcinogenic effectiveness. A tumor-"initiating" dose of UV-B (4–10 wk of daily FS lamp exposures) was rendered *less* effective by subsequent exposures of the mice to UV-A (6 hr/day, F-40 T12BL lamps). The mechanism for this effect is not known. Most tumors induced by a short course (10 wk) of FS lamp exposure grew slowly or regressed, whereas mice exposed for a longer period (30 wk) developed more tumors, and many of those that appeared early grew aggressively. Effects of daily dose fractionation were less clear, and the subject requires further study. These and other variables are being tested in a program designed to yield useful information on the effects of changing spectrum, dose, and dose delivery rates on sunlight-induced cancer.—Natl Cancer Inst Monogr 50: 31–38, 1978.

A small amount of ozone in the atmosphere absorbs UVR that would otherwise reach the earth's surface. In the absence of this ozone, UVR damage to plant and animal cells would make life as we know it impossible. A small decrease in the amount of atmospheric ozone would presumably lead to some increase in such recognized solar UV effects as human skin cancer and in other photobiologic changes, which are even less well known. Part of the study embodied in this report was undertaken as a result of calculations which indicated that nitrous oxides in exhaust gases from a fleet of SST aircraft could photocatalytically decompose some of the stratospheric ozone. More recently, other nitrogenous and certain chlorofluorocarbon materials have also been implicated in potential modification of the ozone layer (*1*).

The amount of atmospheric modification is not predictable with certainty. Furthermore, it is clear that quantitative studies performed to date do not provide data adequate for a reliable extrapolation from hypothetical stratospheric modification to an alteration in skin cancer risk (*2*).

This is partly due to the fact that the action spectrum for carcinogenesis is not known but is assumed to be like that for human skin erythema. Erythema action spectra, in turn, vary with the methods of determination, and the variation is precisely in the spectral region most affected by ozone absorption (*see* "Workshop on Production and Measurement of UV Light"). Mattingly (*3*) has shown that calculated solar UVR sunburn dose rates are extremely sensitive to the assumed shape of the erythema action spectrum and that the action spectrum for a given biologic effect must be accurately measured if the change in that effect due to a decrease in ozone is also to be calculated accurately. It is obvious, but seldom emphasized, that virtually no direct data are available on dose–response or action spectra for human skin UV photocarcinogenesis; furthermore, such data are not likely to come into existence soon.

Blum (*4*) reported extensive observations on experimental photocarcinogenesis using the ears of albino mice. These and numerous studies done by others on haired and hairless mice have proved the value of experimental work on factors that influence the expression of photocarcinogenesis (*5, 6*). Unfortunately, little of this information has a direct bearing on the question raised by the ozone problem, viz., what is the effect of a specified incremental change in the wavelength region of 290–310 nm?

Some of the variables and uncertainties implicit in quantitative models can be tested directly in the laboratory. For instance, the influence of additional short-wave UVR ($\lambda < 300$ nm) on carcinogenic effectiveness can be assessed quantitatively and qualitatively in animals; dose–response relationships can be determined for several wave bands, and the influence of dose delivery (flux, discontinuous exposure, etc.) can be evaluated for simulated sunlight.

In dose–response studies, the range of doses is delivered by variations in either intensity or duration of exposure, but either approach assumes reciprocity, i.e., that the effect is proportionate to cumulative dose regardless of rate or duration of delivery. There is reason to believe that the relationship should hold, at least for some spectra and over fairly narrow ranges. However, few direct tests of time–dose reciprocity in photocarcinogenesis have been attempted, and with the need for repeated exposures for a long time, the question is technically complex and will not be settled quickly.

We are currently testing some of these variables, and our early results are presented here.

Abbreviations: λ = wavelength(s); UVR = ultraviolet radiation; UV-A = 320–400 nm; UV-B = 280–320 nm; EEE = erythema effective energy; BL = black light; J = joule(s).

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[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] Supported in part by Public Health Service contract N01-CP43271 and grant CA11536 from the National Cancer Institute and grant ES00269 from the National Institute of Environmental Health Sciences.

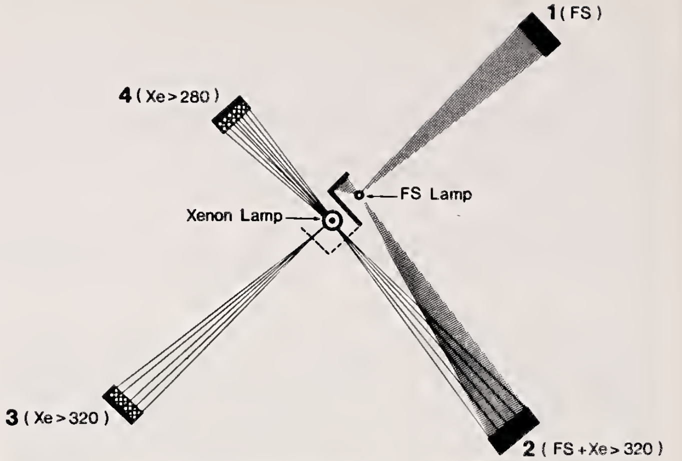
[3] The Center for Photobiology, Skin and Cancer Hospital, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140.

WAVELENGTH INTERACTIONS

We reported (*7*) an experiment that suggested that when two dissimilar light sources were matched for erythema effectiveness, the one emitting more UV-A was more effective in producing skin tumors in hairless mice. The sources also differed in intensity. We subsequently conducted experiments to test possible effects of UV-A and of dose delivery on photocarcinogenesis.

Method (Experiment A)

Two light sources (an FS-40 T12), Westinghouse Sun lamp and a long-arc xenon lamp), plus two optical filters (Schott WG345 and UG11) were used. The FS lamp was the primary source of UV-B (λ < 320 nm), and the xenon lamp (equipped with infrared absorbing inherent filtration) was the primary source of UV-A and visible radiation (λ > 320 nm). The physical arrangement of the lamps is shown in text-figure 1. Five groups of mice (24 in each group) were exposed simultaneously for 2 hours/day, Monday through Friday, for 30 weeks. Group III received only radiation λ greater than 320 nm. Groups I, II, and V received UV-B from the same FS lamp, with varying amounts of UV-A or visible radiation from the xenon lamp; group IV was exposed to UV-B, UV-A, and visible from the xenon lamp (table 1). Group IV was positioned nearer the xenon lamp so that its erythema effectiveness, as determined by a Westinghouse WL767 meter (*8*) was equal to groups I, II, and V (300 J/m² EEE). The 5 source spectra (text-fig. 2) were determined by a Cary-14 spectrophotometer with radiometer accessory (*9*). The techniques for mouse exposures, maintenance, and data collection have been described previously (*10, 11*). Briefly, SKH hairless-1 mice were housed and irradiated in multiple unit isolation cages. Each mouse was examined weekly, and any changes in skin appearance were noted on a diagram. The course of tumor appearance was plotted as incidence (affected mice/survivors) or tumor yield (total tumors/survivors) versus time. The arc sin transform was used to fit a curve to incidence data, and a second-order polynomial was used to fit a curve to tumor yield data (*12*).

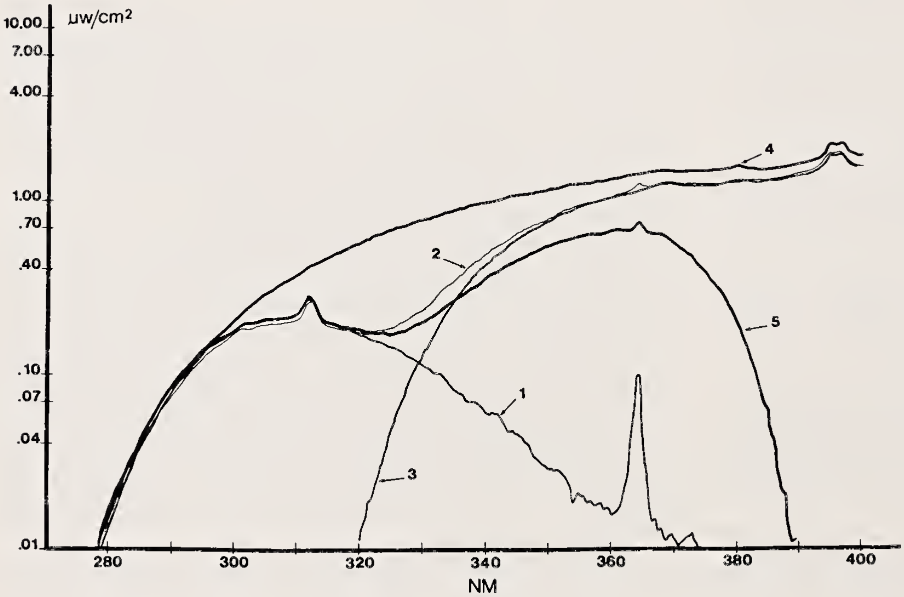


TEXT-FIGURE 1.—Schematic diagram of lamp and filter arrangement used in expt A; two vertical lamps shown as viewed from above. Positions #1–4 correspond with animal groups I–IV. For simplicity, position 5 is not shown; 5 was identical to 2, but with the UG-11 filter (UVR transmitting, visible light absorbing) in the beam.

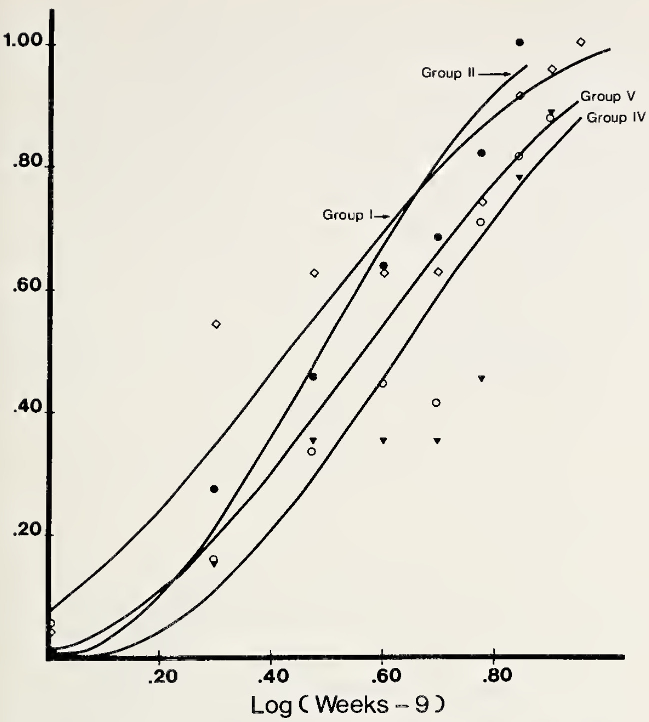
TABLE 1.—*Light sources, filters, and source spectra for expt A*

Group	Light source		Added filter		Major wave bands included
	FS	Xe			
I	FS	—	—	—	UV-B
II	FS	Xe	WG345	—	UV-B, UV-A, visible
III	—	Xe	WG345	—	UV-A, visible
IV	—	Xe	—	—	UV-B, UV-A, visible
V	FS	Xe	WG345	UG11	UV-B, UV-A

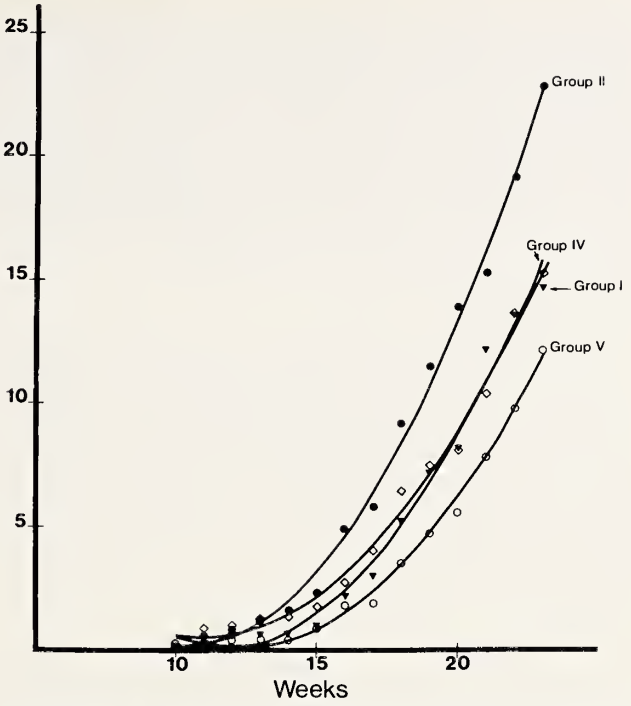
For all studies in this report, incidence is calculated on a cumulative basis, i.e., at a given time, an animal is considered "affected" if it has ever had at least 1 tumor. Likewise, tumor



TEXT-FIGURE 2.—Source spectra for the lamp positions shown in text-figure 1 (expt A) showing radiation detected at animal cage surface. *Numbered curves* correspond with positions in text-figure 1.



TEXT-FIGURE 3.—Tumor incidence (affected mice/survivors vs. time) in expt A.



TEXT-FIGURE 4.—Tumor yield (tumors/survivors vs. time) for expt A.

yield involved all tumors that have ever appeared, irrespective of tumor longevity (*see* "Discussion" section).

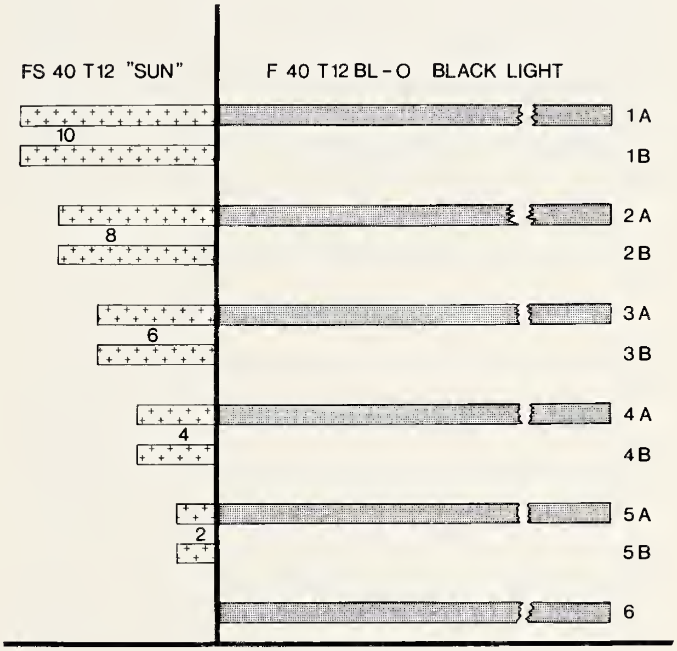
Result (Experiment A)

Animals in group III (λ > 320 nm only) developed no skin tumors. In all other groups, tumors began to appear after about 10 weeks of exposure (text-fig. 3). Expressed as incidence, no treatment spectrum appears significantly more effective than any other. Tumor yield appears higher for group II, which received added (long-wave) radiation from the xenon lamp (text-fig. 4). However, group IV did not show greater tumor yield than group I (text-fig. 4). In a replicate experiment (not shown) group II had a slightly lower tumor yield than the other groups. Thus the addition of simulated sunlight (UV-A, with or without the visible component) does not seem to enhance the carcinogenic effectiveness of 280- to 320-nm UV-B.

The next experiment also tested UV-B and UV-A interaction but the exposures were sequential.

Method (Experiment B)

One hundred thirty-two SKH hairless-1 mice were randomized into 11 groups of 12 each and irradiated in a scheme indicated in text-figure 5. The aim was to expose groups to varying amounts of UV-B, followed either by no treatment or by exposure to UV-A. The design was analogous to an initiation–promotion experiment, with all applications of initiator (UV-B) completed before the application of the test promoter (UV-A) were begun.



TEXT-FIGURE 5.—Design for expt B. Mice were exposed to FS sun lamps for 5 min daily for 0–10 wk; subgroups were then left untreated (B) or exposed 6 hr daily (A) to black light (λ > 320 nm).

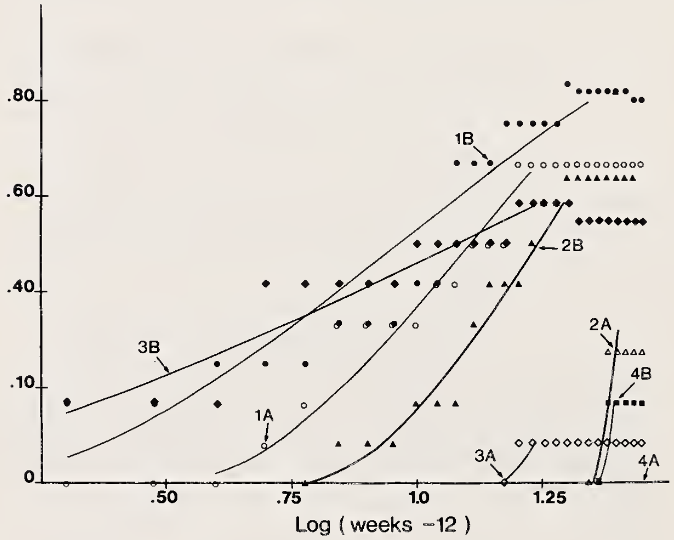
The UV-B source was a bank of FS-40 T12 Westinghouse sun lamps to which mice were exposed for 5 minutes daily, Monday through Friday, for 2, 4, 6, 8, or 10 weeks. As measured by the WL767 meter, the daily skin dose was 300 J/m² EEE. Twelve mice from each UV-B dose level were subsequently exposed for 6 hours daily to a bank of F-40 T12BL lamps, filtered through 6-mm glass (*13*). In addition, 12 mice were exposed to the BL lamps only (group VI). The daily UV-A dose was approximately 4.32 × 10⁴J/m² centered at 365 nm, calculated from an International Light IL600A Research Photometer.

Result (Experiment B)

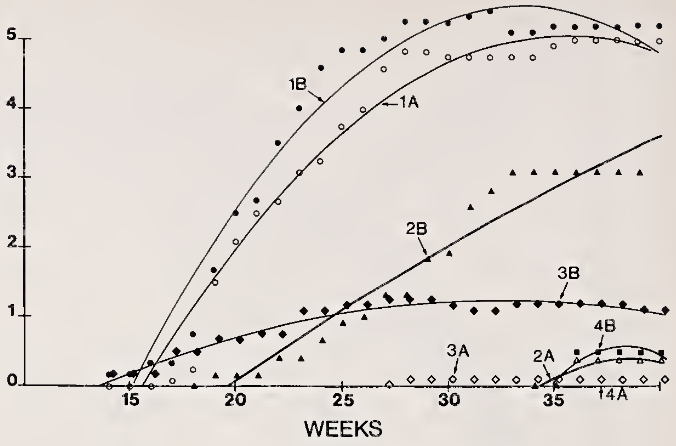
None of the mice receiving 0 or 2 weeks of UV-B (groups V, VI) developed skin tumors, but some in all other groups were affected, with the greatest effect in the mice accumulating the most UV-B exposure (text-figs. 6, 7). In all 4 groups, the effects (tumor incidence and yield) were *lower* in the subgroups receiving subsequent UV-A exposure. The tendency for tumors to persist and grow was no greater in the groups receiving added UV-A.

TIME–DOSE RELATIONSHIPS

One of our pilot studies was based on mice receiving equal daily UVR doses, delivered either as an intense 5-minute exposure, or as attenuated 50- or 500-minute exposures. The inflammatory response during the first 2 weeks and the tumor data indicated that effectiveness of the three schedules was not equal but was *inversely* proportional to flux (unpublished data). To achieve the desired beam attenuation, we used lamp-to-skin distance as a variable. UVR detector geometry poses some problems in making direct comparisons in this kind of system. The study was repeated but based on neutral density filtration. In the meantime, another study on attenuation was completed, in which we used a design involving fractionation of the daily dose.



TEXT-FIGURE 6.—Tumor yield (expt B). Groups 5 and 6 had no tumors and are not shown. *Note* that in each pair of subgroups, the UV-A-exposed member was less affected.



TEXT-FIGURE 7.—Tumor incidence (expt B).

Method (Experiment C)

Two groups of 24 SKH hairless-1 mice were exposed for 5 minutes daily (Monday through Friday) to a bank of FS-40 T12 sun lamps (300 J/m² EEE). The exposure given to group I was delivered as one continuous 5-minute period; group II was exposed for 1-minute periods, 1 hour apart (for a span of about 4 hr each day). We kept the daily doses arithmetically identical by controlling the light source with a timer and a preset accumulating energy detector.

Result (Experiment C)

Tumors began appearing in both groups after about 10 weeks. Following an initial lag, tumors accumulated more rapidly in group II than in group I (text-figs. 8, 9), although the absolute difference remained small to the end of the experiment.

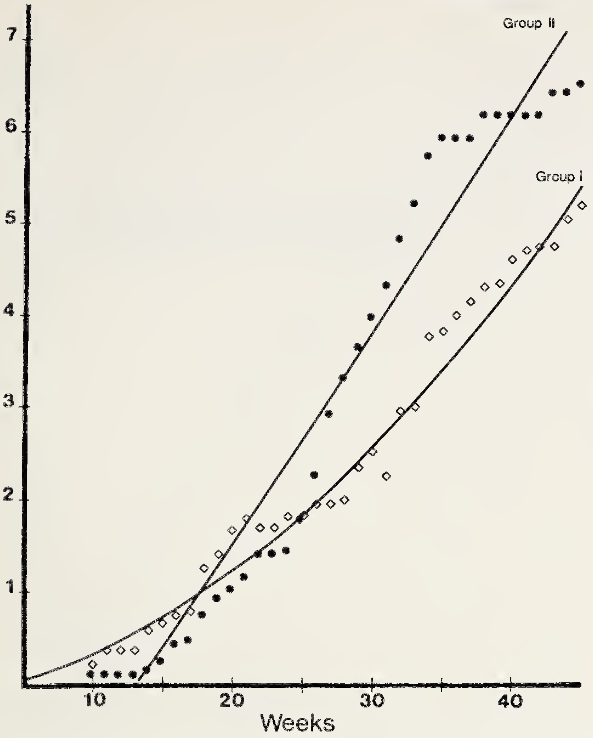
Most tumors induced in hairless mouse skin by a single traumatizing dose of UV grow slowly, if at all, and eventually regress (*14*). In contrast, tumors in chronically exposed mouse skin continue to grow. Experiment D illustrates a third situation, i.e., repeated exposures of skin, with daily doses low enough to avoid destructive skin changes but with the treatment discontinued before the first tumors were clinically visible (*15*).

Method (Experiment D)

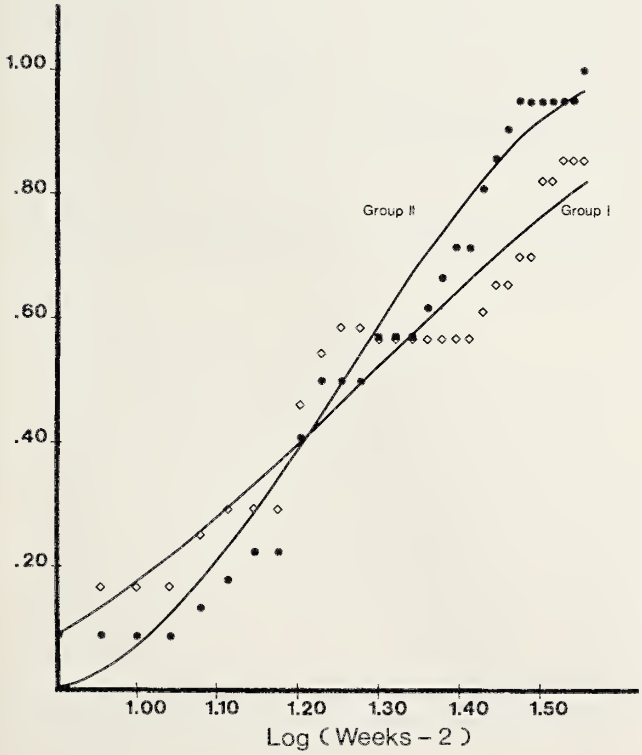
Two groups of SKH hairless-1 mice were exposed for 5 minutes daily (Monday through Friday) to a bank of FS-40 T12 lamps (300 J/m² EEE). Group I (48 mice) was exposed for 30 weeks, whereas group II received the same treatment for only 10 weeks.

Result (Experiment D)

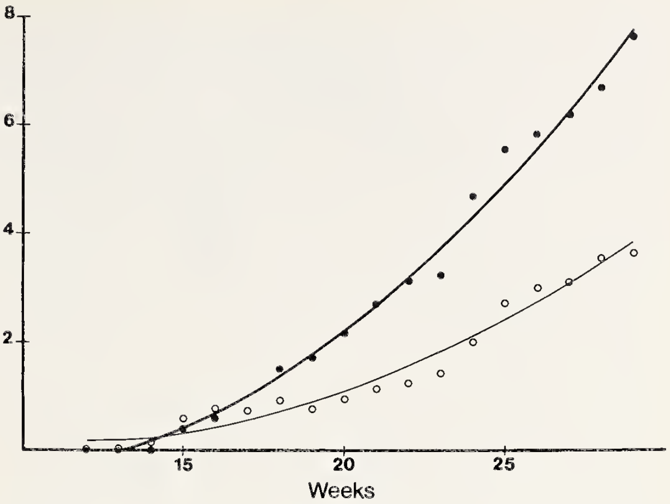
Tumors began to appear in both groups at about 12 weeks; incidence and yield increased in both groups throughout the observation period. The two were indistinguishable for several weeks, but subsequently diverged, with group I increasing more steeply (text-figs. 10, 11). Tumors in group I tended to persist and grow, but many in group II remained small or regressed and disappeared.



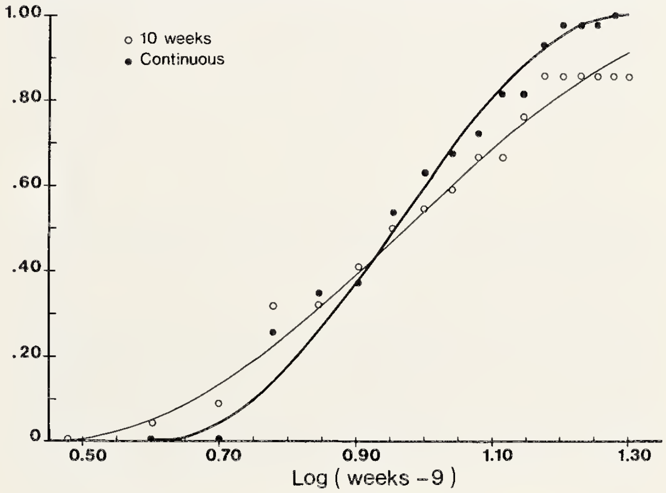
TEXT-FIGURE 8.—Tumor yield (expt C). Group I received one 5-min exposure daily; group II received five 1-min exposures daily, for a period of approximately 4 hr.



TEXT-FIGURE 9.—Tumor incidence (expt C).



TEXT-FIGURE 10.—Tumor yield (expt D). Mice were irradiated 5 min daily for 10 wk *(open circles)* or for 30 wk *(closed circles).*



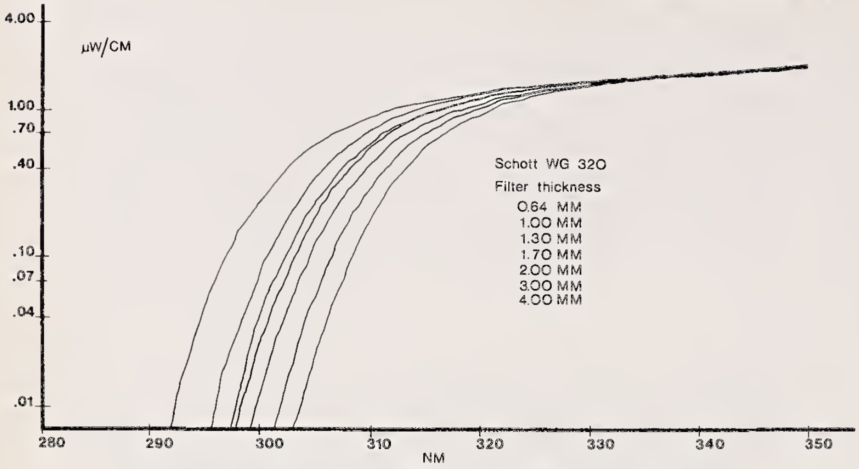
TEXT-FIGURE 11.—Tumor incidence (expt D).

VARIATION OF SOLAR UV-B SPECTRUM

Small changes in atmospheric ozone content could lead to significant modification of ground-level UVR, but the change would be confined to a narrow spectral band around λ 300 nm. We developed a system for generating UVR with a broad spectrum (xenon) source, modifying the beam at selected wavelength intervals by varying ozone or other filter materials, measuring and recording the resultant spectra, and irradiating biologic specimens (*see* Workshop section). Several studies on photocarcinogenesis were designed to explore the influence of spectral effects in the 300-nm region. Preliminary data on the first of these studies are presented here.

Method (Experiment E)

Six groups of 72 SKH hairless-1 mice were exposed simultaneously to a long-arc xenon lamp. The exposure lasted 2 hours/day, Monday through Friday, for 40 weeks. Six WG 320 Schott filters (0.64 to 3.00 mm thick) were used to establish 6-source spectra, one for each group of mice (text-



TEXT-FIGURE 12.—Source spectra, by long-arc xenon lamp and Schott filters; 4.00-mm filter was not used in animal studies.

TABLE 2.—*Readings of radiation reaching cages of SKH hairless-1 mice*

Schott WG 320 filter thickness, mm	Relative intensity[a]	
	R-B meter	WL767 meter
0.64	3.19	3.92
1.00	2.28	2.85
1.30	2.07	2.28
1.70	1.77	1.97
2.00	1.41	1.58
3.00	1.00 [a]	1.00 [a]
4.00	0.71	0.82

[a] The R-B meter and the WL767 meter readings were normalized at the thickest filter used in expt E (3.00 mm).

fig. 12). The radiation reaching the cage surface was continuously monitored and recorded for each group with the use of a multiple-unit R-B detector (*16, 17*) built for the purpose. Readings were also taken with a Westinghouse WL767 zirconium phototube (table 2). Detailed spectroradiometric (Cary-14) readings (text-fig. 12) were made approximately weekly.

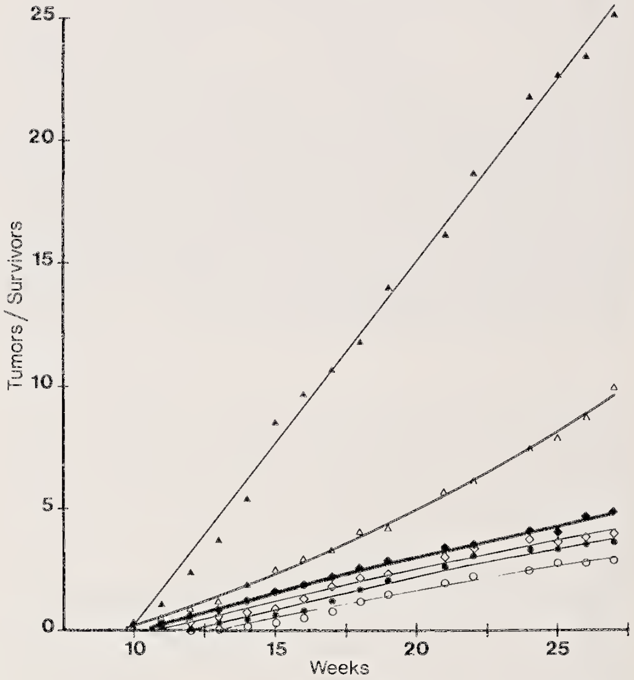
For comparison purposes, the WL767 value of 1 (table 2; 3-mm filter) is 119 J/m² EEE/daily dose; the analogous R-B reading is 85 counts.

Result (Experiment E)

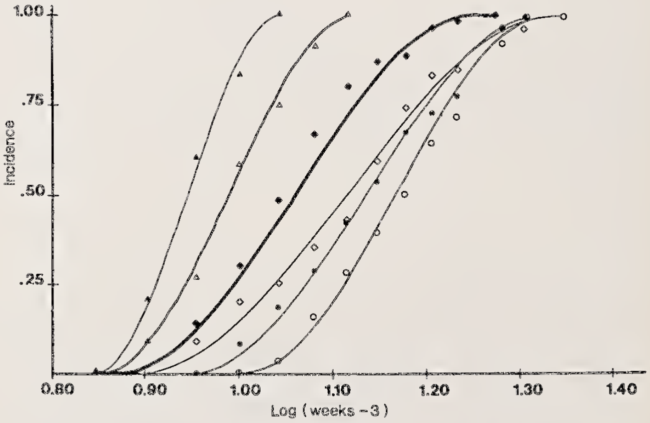
Tumors appeared earliest and with greatest frequency in inverse relation to filter thickness. Tumor yield data were adequately described by second-order polynomial expressions (text-fig. 13), and curves were readily fitted to incidence data by arc sin analysis (text-fig. 14). The smoothness and regular displacement of the curves would suggest that incremental additions of shorter wavelengths (λ <310 nm) in the exposure spectrum lead to detectable increase in photocarcinogenesis. With all filter thicknesses (i.e., with all spectral qualities) a close similarity occurred between time to 0.5 incidence and time to an average yield of 1 tumor per survivor (text-fig. 15).

DISCUSSION

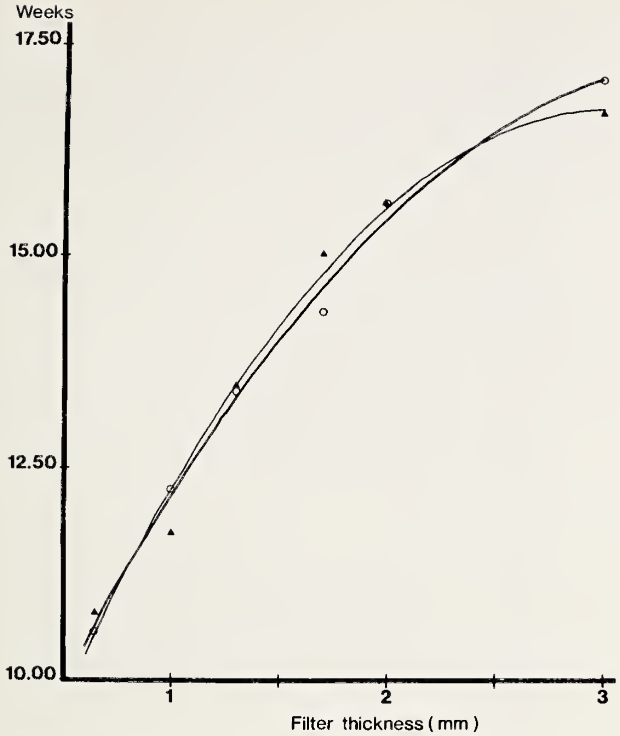
Even in frequently and carefully examined experimental animals, the precise time of onset of a given skin tumor is not



TEXT-FIGURE 13.—Tumor yield vs. time (expt E). The groups correspond, *left* to *right*, with respective spectral sources in text-figure 12.



TEXT-FIGURE 14.—Tumor incidence vs. log time (expt E).



TEXT-FIGURE 15.—Expt E. Time to 0.5 incidence (▲) is closely related to time to 1.0 tumor yield (○) for all filter thicknesses.

known. At the stage when a tumor can be distinguished from other skin excrescences (∾ 0.5 mm diameter), it already contains at least several hundred thousand cells. At the other end of the scale, lethality is an unambiguous end point but has little else to commend it as an effectiveness criterion, since many animals accomodate themselves to their tumors, and those who do succumb yield little other useful information. Between the two extremes of first minimally perceptible tumor and lethality, the criteria for tumor presence are arbitrary (size, duration, morphology, behavior, multiplicity).

Analogously, human tumors become significant when they inflict a social or economic burden, or interfere with function; this would be true even if tumors did not involve lethality. Traditionally, however, the impact of tumorigenesis tends to be weighted in terms of cancer death rate.

Apparent skin cancer rates in the United States are influenced by numerous factors, including variable likelihood of tumor recognition and reporting among clinicians, regional differences in patients' awareness and concern, and whether the compiling registry will accept information on multiplicity of tumors in patients already affected. An important point here is that this multiplicity may not be counted in the summed "new cases per year," but it still would pose a social and economic burden. Few clinical data are available from which to estimate the magnitude of this tendency to underestimate tumor burden. Analytic methods for dealing with clinical and experimental tumor data can be at once primitive and complex; they will continue to evolve with experience.

We presented a partial analysis of data from several experiments. Because the natural history of each tumor is recorded, other analyses, including the influence of tumor loss and animal mortality, will be made as later data become available. The criteria used in this report are tumor *incidence* and *yield*. Because these terms have a number of possible connotations, our use will be explicated here.

Tumor incidence is the proportion of all surviving animals that have or have had at least 1 tumor; for this measure, spontaneous loss of tumors is ignored. Since animals are removed from the calculation after their death, small apparent decreases in incidence may be observed, especially in small groups. Generally, however, the shape of the incidence versus time curve is sigmoid, though somewhat skewed on linear coordinates. With a "linearizing" transformation of incidence (we used the arc sin transform), it is apparent that incidence is more directly related to a function of the logarithm of time. The absolute values of the time scale are critically important to this logarithmic transformation, and the concept of "zero time" is never clear when treatments are prolonged or when observations include an apparent "latent period" associated with detection of a macroscopic lesion. For this reason, the time dimension is obtained from a convenient arbitrary zero (start of experiment) with a correction term fitted empirically to minimize the residual variance of the relation:

$$\sin^{-1}(2P-1) = a + b \log (T-k)$$

where P is the observed incidence, T is weeks after the start of the experiment, k is an integer between 0 and (T − 1), and a and b are constants. Since k is generally considered to be an experimental characteristic independent of treatment (*12*), its value has been derived from combined data of all experimental groups. For the data presented in text-figures 6 and 7, k = 12. However, in unrelated experiments, we noted that k may be strongly treatment related, which suggested that for larger group sizes, individual k values for each group would be more descriptive.

Yield is the average number of tumors present on surviving animals. In situations involving constant treatment, it is frequently an accelerating function of time, which in such cases is an analog of cumulative dose. When treatment is discontinued, however, yield as a function of time often tends to a maximum; if the data consist of net numbers of tumors present, values may subsequently decline as spontaneous (or other) losses outpace further appearance of tumors. In our experience, yield data are usually well represented as second-order functions of time. Continued treatment is associated with positive (or zero) values of the second-order coefficient, whereas discontinued treatment produces negative second-order coefficients.

In the experiment described in text-figures 6 and 7, the UV-B stimulus was always terminated at week 10. In each instance, the net tumor yield reached a maximum and subsequently plateaued or began to decline. Subsequent treatment with UV-A not only failed to prevent the decline but actually decreased the yield associated with each cumulative dose of UV-B. At least in the quantities used, UV-A neither initiated nor enhanced the growth of tumors (expt B). In addition, UV-A as used did not appear to act as a promoter of (latent)

UV-B-initiated tumors. In fact, the apparent inhibition of carcinogenesis was unexpected, and in the absence of further information, the mechanism is not obvious.

Although experiments A and B relate to UV-A effects, they differ significantly in test method. In experiment A, we used the wave bands approximately in the proportions found in summer sunlight, delivered simultaneously. Experiment B involves more prolonged exposures to UV-A, delivered after the UV-B exposures had ceased.

When daily UVR exposures ceased before any skin lesions were clinically visible, tumors developed subsequently, and continued to appear for at least several months; however, few grew substantially and most regressed (expt D). Tumors developing in mice continually irradiated tended to persist and grow. Thus UV-B not only initiated but enhanced the growth of skin cancers in hairless mice.

We are left with uncertainty on the question of the limits of time–dose reciprocity. Earlier data had shown an inverse relationship between UV-B flux and carcinogenic effectiveness. The present study (expt C) suggests that alteration of dose delivery by fractionation of the daily dose produces an increased lag period, followed by a steeper response slope, but both are small and perhaps insignificant. Further research on this question is under way.

Experiments A and E are complementary approaches to the question of spectral effects. In A, the lower end of the emission spectrum ($\lambda < 300$ nm) is kept constant, and comparisons are based on variations in the UV-A and visible components. In E, the UV-A and visible are held constant, with the lower end varied stepwise. In the presence of a constant spectrum greater than 330 nm, incremental additions of wave bands at the shorter end of the spectrum make sizable differences in carcinogenic effectiveness (expt E). Taken together, experiments A and E indicate that the increased effectiveness is a direct function of the UV-B component, as distinct from an interactive process dependent on or strongly influenced by the presence of UV-A.

In the usual UVR dose–response study, a dose axis is generated as an analog of dose delivery time, because the spectrum and the dose rate are constant. In contrast, the altered spectra that constitute the intermediate variable in the present ozone problem provide no basis for estimating "dose," since the relative effect of different spectral regions is unknown. We have generated a family of "ozone path-response" curves. The next logical step in understanding this relationship is to determine the meaning of "dose" for various input spectra, by delivery of a series of doses with each spectrum. Using the same irradiation system, we are also testing the limits of reciprocity, i.e., whether the effect is proportionate to cumulative dose regardless of rate, duration, or time distribution of delivery.

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Photosensitized Reactions and Carcinogenesis[1, 2]

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ABSTRACT—We present data from experiments designed to investigate the role of DNA interstrand cross-links induced by exposure to 8-MOP plus UVR and skin carcinogenesis. 8-MOP was administered topically to two strains of hairless mice, SKH:hairless-1 and HRS/J/An1, which were then exposed to UV light sources with emission in the range of 1) 300–400, 2) 320–400, and 3) predominantly 365 nm. We found no strain dependency for DNA cross-link production, but a marked strain-dependent difference in tumor susceptibility was noted. Only a small strain-dependent difference occurred in tumor incidence when TPA was administered after exposure to 8-MOP and 320–400 nm. These results suggest that the events concerned with tumor promotion are dependent on strain. Because the most effective tumorigenic wavelength spectrum was 300–400 nm, we investigated the possibility of interaction between lesions induced by the 300- to 320-nm wavelengths and the psoralen photoadducts. In the course of this experiment, we found that the tumorigenic effect was also dependent on the time interval between exposures to 8-MOP plus 365-nm light.—Natl Cancer Inst Monogr 50: 39–43, 1978.

This paper is concerned with photosensitization by 8-MOP, one of a group of compounds called psoralens or furocoumarins. Table 1 shows the information that was available for tumorigenic effects of exposure of mice to psoralen and UVR when the experiments reported here were considered. In 1972, Forbes et al., using a solar simulator as the light source, were extending the studies of Urbach (*4*), and their results were reported subsequently (*5*). This later study was important because it established that combined treatment with topical 8-MOP and a broad spectrum light source produced epidermal carcinomas in hairless mice. Furthermore, these authors found pretreatment with anthracene produced acute photosensitization but did not increase tumor incidence.

When the studies in the 1950's on the effects of the combined treatment of psoralen and UVR were done, there was no understanding of the formation and nature of the photoadducts. In 1967, it was shown that furocoumarins, with 365-nm irradiation, photoreact with pyrimidine bases to form monofunctional adducts involving either the 3,4-pyrone or 4′,5′-furan double bonds of the furocoumarin and the 5,6-double bond of the pyrimidine base [(*6, 7*); also *see* reviews (*8, 9*)]. With irradiation at 365 nm, the photoadducts can photoreact further to form bifunctional adducts or interstrand DNA cross-links (*10, 11*). The introduction of methods of assaying the photoproducts (*11, 12*) suggested the possibility of examining the quantitative relationship between these photoproducts and tumor induction.

To establish a quantitative relationship between a molecular lesion and subsequent tumor formation, one must determine the dose–response relationships for the induction of the molecular lesion and the tumors. Establishment of a causal relationship requires the further step of demonstrating that the removal or repair of the induced molecular lesion results in a reduction in tumor incidence. Unfortunately, neither of these approaches appeared possible, so an indirect approach was chosen to investigate the relationship of psoralen- plus UVR-induced DNA cross-links and tumor induction with two strains of hairless mice and three light sources. The aim was to use dose levels of the combined psoralen–UVR treatment that induced equivalent amounts of DNA cross-links and other biologic effects, such as cell killing and changes in cell proliferation in the two strains. If the psoralen–DNA cross-link was the tumorigenic molecular lesion, then mice of the two strains and in the groups exposed to the three light sources should show similar tumor incidences.

MATERIALS AND METHODS

Experimental animals.—We used females of two strains of hairless albino mice: HRS/J/An1, an inbred strain of mice cesarean-derived and maintained as an outbred stock at this Laboratory, and SKH:hairless-1, an outbred stock of animals (*13*) supplied by Dr. P. D. Forbes of Temple University.

Light sources.—We reported the emission spectra for the three light sources (*14*): 1) Magnaflux mercury vapor lamp (365 nm); emission consists of a high intensity line at 365 nm with less than 1% of the total fluence at 334 and 405 nm. 2) Westinghouse F40BLB fluorescent lamp (300–400 nm, Westinghouse Lamp Division, Bloomfield, N.J.); emission is a continuous spectrum between 300 and 400 nm with about 1% of the total fluence below 320 nm. 3) Filtered Westinghouse F40BLB fluorescent lamp (320–400 nm); filtering with glass removes all measurable emission below 320 nm. The fluence rates from the three sources were 2.6, 4.6, and 3.1 watts/m^2, respectively.

Radionuclide labeling of epidermal DNA.—1) In the cross-

Abbreviations: 8-MOP = 8-methoxypsoralen; TPA = 12-*O*-tetradecanoylphorbol-13-acetate; UVR = ultraviolet radiation; MED = minimum erythema dose; J = joule(s).

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[2] Supported in part by the U.S. Energy Research and Development Administration and by contract T-3568E with the National Aeronautics and Space Administration.

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[6] We gratefully acknowledge the assistance of Mrs. B. Ann Sedita, Mrs. Kathy A. Rettman, and Mrs. Cheryl A. Bedwell.

TABLE 1.—*Skin cancer studies with psoralens*

Route of administration	Type of UVR, nm	Tumor response	References
ip	Up to 320	Increased	*(1)*
Dietary		Decreased	
ip	∾ 320–400	Increased	*(2)*
Dietary		Increased	
Oral	Up to 320	No effect	*(3)*
Topical	275–360	Advanced appearance	*(4)*
ip		" "	
Oral		Not significant	

link determinations, female mice, 12 weeks of age, received 4–6 ip injections of 75 μCi of [^{3}H]thymidine (8–11 Ci/mmol; New England Nuclear, Boston, Mass.) at 6-hour intervals.

2) For our proliferation studies, mice were killed 45 minutes after a single ip injection of approximately 1.0 μCi [^{3}H]thymidine/g body weight (0.36 Ci/m mole). Autoradiographs were prepared by the dipping technique with Eastman Kodak NTB Emulsion (Eastman Organic Chemicals, Rochester, N.Y.).

Biologic assays.—The assays of DNA cross-links were based on techniques described by Cole *(11)* and Ben-Hur and Elkind *(12)*, and the method of DNA isolation was described *(14)*.

The MED was determined at 48 hours after exposure to graded fluences of UVR 1 hour after the topical application of 20, 50, 150, 250, and 500 μg 8-MOP (Paul B. Elder Co., Bryan, Ohio) or 2 hours after 50, 250, 500, and 750 μg 8-MOP were administered by intubation. Erythema was graded visually by at least two observers on replicates of at least 4 mice per dose level. For both routes of administration, the MED is the fluence that induces barely perceptible erythema at 48 hours.

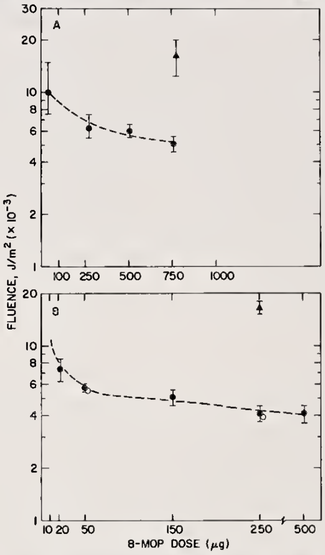
For the epidermal cell population studies, all determinations of cell density and proliferative activity were made on the interfollicular areas. The numbers of nucleated cells and [^{3}H]-labeled cells were determined per microscopic field from replicate measurements with a minimum of 1,000 basal cells scored for each animal.

In our tumor studies, mice were exposed to UVR 45–60 minutes after topical application of 250 μg 8-MOP dissolved in reagent grade absolute ethyl alcohol and distributed over the skin of the back. The combined treatment was given five times/week for 24 weeks, and the exposure groups were: 1) 300–400 nm, 1.25×10^3 J/m^2; 2) 320–400 nm, 1.25×10^3 J/m^2; and 3) 365 nm, 5.5×10^3 J/m^2. In tumor promotion studies, groups of mice were treated with 250 μg 8-MOP and 320–400 nm UVR 1.25×10^3 J/m^2 three times/week for 6 or 12 weeks, or five times/week for 7 weeks, and subsequently with 10 μg TPA three times/week for 26 weeks. Only histologically confirmed epidermal carcinomas have been used in the tumor data analysis. The time of appearance of tumors was taken as the time tumors (subsequently confirmed histologically) approximated 1.0 mm in size. We calculated the cumulative percentage of tumor-bearing mice by using an actuarial adjustment for intercurrent deaths or removal of mice from the experiment.

RESULTS

A number of problems are encountered in tumor studies that entail repeated exposures to UVR and treatments with chemicals. First, the route of administration of the chemical must be suitable. In combined treatment, in which the biologic activity is dependent on both the dose level of 8-MOP and the fluence of the UVR, it was necessary to establish the level of 8-MOP with which the effects of the combined psoralen–UVR treatment would depend only on the fluence and the wavelength spectrum of the UVR.

The data in text-figure 1 and table 2 illustrate that with the topical application of 8-MOP, the erythemogenic effect became dependent solely on the fluence of UVR at doses of about 250 μg. About five times the dose of 8-MOP had to be given by the oral route for comparable MED and DNA cross-link induction with 320- to 400-nm light. When the oral



TEXT-FIGURE 1.—A) Influence of the oral dose of 8-MOP on the fluence of UVR required for induction of minimal erythema in skin of SKH:hairless-1 mice. Mice were intubated with varying concentrations of 8-MOP in 0.5 ml cottonseed oil, and 2 hr later, groups of mice were exposed to graded fluences of 320–400 nm (●) and a single fluence of 365 nm (▲). B) The influence of the dose of 8-MOP applied topically in 0.1 ml ethyl alcohol spread over ∾ 15 cm^2 of dorsal skin to the minimal erythema induced by exposures to graded fluences of 320–400 nm to SKH:hairless-1 (●), or to HRS/J/Anl (○), and exposure of 365 nm to SKH:hairless-1 (▲). Mice that received 8-MOP or UVR alone were used as controls. *Bars* represent the range of effective fluences observed in replicate experiments with 4 mice/group in each.

TABLE 2.—*Relative efficiencies for erythema and cross-link induction after a single treatment with 8-MOP and 320–400 nm*

Dosage of 8-MOP, μg	Administration	Cross-link induction, m^{2}J^{-1} dalton^{-1} $\times$ 10^{-13}	MED, 320–400 nm, J/m^2
500	Topical	2.6	4×10^3
250	"	2.8	4×10^3
150	"	2.3	5×10^3
750	Oral	2.8	5×10^3

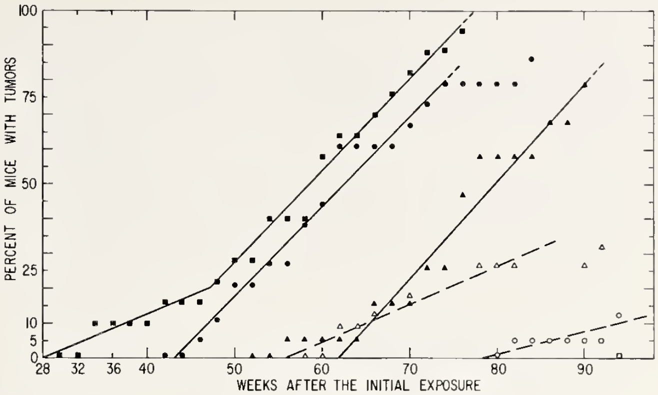
8-MOP dose was adjusted to give an erythemal response comparable to that observed after combined treatment with 8-MOP applied topically, the efficiency for DNA cross-link induction was the same for the two routes of administration. The MED was not dependent on the strain of mouse but was wavelength dependent (text-fig. 1). Previously, we found that DNA cross-link induction was independent of strain but dependent on the UVR spectrum (*14*).

Topical administration of 250 μg 8-MOP was chosen for all the tumor studies. Because of the low efficiency for cross-link induction with 365 nm, 500 μg of 8-MOP was used in the bifunctional adduct assays.

A second problem with studies of UVR induction of skin tumors is dosimetry. It is difficult to obtain dose–response curves for induction of tumors with fractionation regimes. For example, if initial exposures result in damage and subsequently hyperplasia, the effective dose incurred from later fractions is altered.

Assays of erythema, cell killing, cell proliferation, and DNA cross-links were used to estimate biologically equivalent fluences for the three UVR spectra. After 6 weeks of 5 exposures/week to the fluences used for the tumor studies, the basal cell population had been reduced, but the rate of proliferation had been increased from control levels (table 3). The fluences used had induced similar numbers of cross-links. Thus the exposure regime that we chose met the following criteria: 1) The molecular lesion of interest was induced in an appreciable quantity; 2) although cell killing occurred, a shortened cell cycle of the survivors increased the proliferation rate and balanced the cell loss; and 3) the basal cell population, although reduced, was stable and no epidermal breakdown occurred over a prolonged exposure period.

In the SKH:hairless-1 mice, combined treatment with 8-MOP and exposure to 320–400 nm UVR resulted in cumulative incidence of skin tumors of about 80% (text-fig. 2). The same irradiation without 8-MOP caused no tumors. These findings show that 1) photosensitization with 8-MOP results in tumor induction with exposure to near-UV, 2) the fact that the combined 8-MOP and 320–400 nm UVR produced similar amounts of DNA cross-links in both strains but different tumor incidences suggests that no simple quantitative relationship exists between psoralen–DNA cross-links and cancer induction. Alternatively, the strain-dependent differences are sufficiently great to confound any relationship between the psoralen–DNA cross-links and cancer induction. The production of erythema by the combined treatment showed similar dose responses in these two strains with different tumor yields. We also found that, although the fluences were adjusted to produce similar biologic effects, including psoralen–DNA cross-links, the tumor incidences were dependent on the UV spectrum (text-fig. 2). The 300–400 nm was the most effective tumorigenic spectral range in the SKH:hairless-1 mice, but it produced no tumors in the HRS/J/Anl mice.

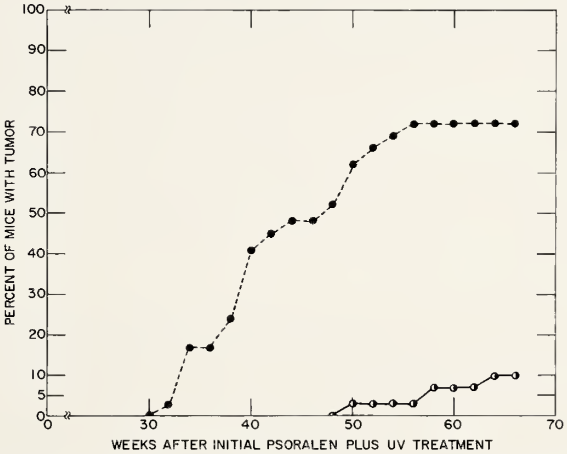


TEXT-FIGURE 2.—Cumulative percent of SKH:hairless-1 mice with 1 or more epidermal carcinomas induced by fractionated exposure (five times/wk, 24 wk) to a daily combined treatment with 250 μg 8-MOP followed at 60 min by either: 1.25 × 10^3 J/m^2 300–400 nm (■); 1.25 × 10^3 J/m^2 320–400 nm (●); or 5.5 × 10^3 J/m^2 365 nm (▲). The corresponding *open symbols* indicate data for HRS/J/Anl mice. These data were previously reported in a different form *(15)*.

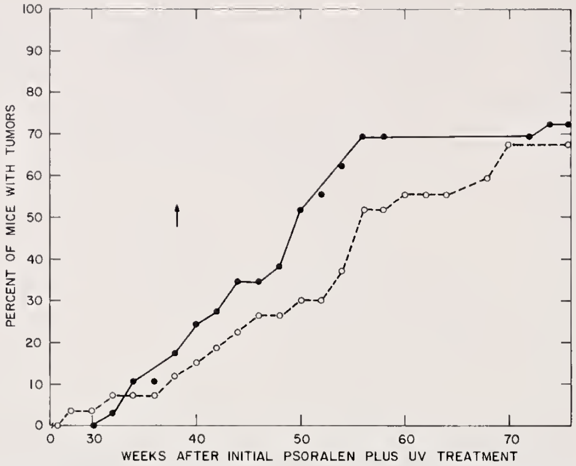
TABLE 3.—*Effects of UVR + 8-MOP on HRS/J/Anl mice*

Treatment, 8-MOP	UVR exposures, J/m^2	No. of exposures at 5/wk	Epidermal changes	
			Basal cell density	Labeled cells/field
—	—	—	18	3
250 μg	1.25 × 10^3 (300–400 nm)	30	12	7
"	1.25 × 10^3 (320–400 nm)	30	12	9
"	5.5×10^3 (365 nm)	30	12	9

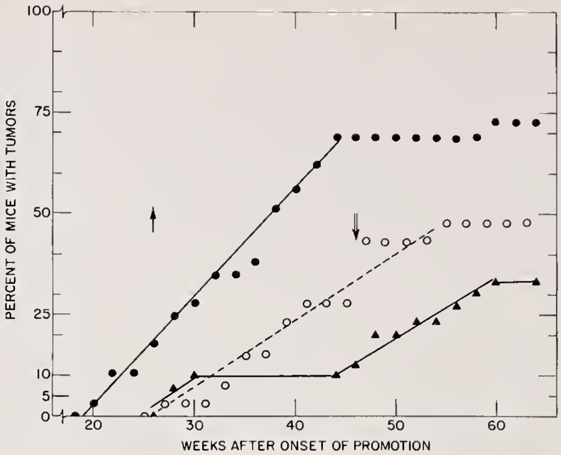
The results in text-figure 3 depict that the tumor initiation events caused by the combined 8-MOP and UVR treatment can be promoted by TPA. The tumor incidence with 3 exposures/week to 8-MOP plus 320–400 nm for 12 weeks was markedly less than with 5 exposures/week (and also for 3 exposures/wk; unpublished data) for 24 weeks. However, when promotion with TPA was started after 12 weeks of 3



TEXT-FIGURE 3.—Cumulative percent of SKH:hairless-1 mice with 1 or more epidermal carcinomas induced by fractionated exposures (three times/wk, 12 wk) to a daily combined treatment of 250 μg 8-MOP and 1.25×10^3 J/m^2 320–400 nm without (◐) or with (●) treatment with 10 μg TPA (three times/wk, 26 wk) given after the end of the exposure regimes.



TEXT-FIGURE 4.—Cumulative percent of mice with 1 or more epidermal carcinomas induced by fractionated exposures (three times/wk, 12 wk) to a combined treatment of 250 μg 8-MOP and 1.25×10^3 J/m² 320–400 nm followed by 26 wk of applications of 10 μg TPA three times/wk; SKH:hairless-1 mice (●), and HRS/J/Anl mice (○). End of period of TPA treatments is indicated by *arrow.*



TEXT-FIGURE 5.—Influence of fractionation on the tumorigenic effects of psoralen plus UV exposures in SKH:hairless-1 mice. Cumulative incidence of mice with epidermal carcinomas from groups of 30 animals, topically sensitized with 250 μg 8-MOP and exposed to 1.25×10^3 J/m² 320–400 nm UVR, three times/wk for 6 (▲) or 12 (●) wk and five times/wk for 7 wk (○). From the week after the termination of psoralen plus UVR treatments, all groups received 10 μg TPA topically three times/wk for 26 wk. End of the initial period of TPA treatment is indicated by *single-bar arrow.* After a 20-wk interval, 10-μg TPA applications were resumed (three times/wk, 26 wk) in 2 of the experimental groups (○, ▲), as designated by the *double-bar arrow.*

exposures/week to 8-MOP and 320–400 nm, the cumulative tumor incidence was similar to that found with 24 weeks of the same combined treatment but without subsequent promotion with TPA. These findings indicate that the later exposures in the 24-week regime are associated with expression of the tumors.

When TPA treatment followed the 8-MOP–UVR exposures, the tumor response in the two strains was similar (text-fig. 4); both the strain-dependent differences in tumor incidence and the time of tumor appearance were much less marked than without TPA (text-fig. 2). In SKH:hairless-1 mice, the tumor incidence is comparable in the group receiving 5 exposures of 8-MOP plus UVR per week for 24 weeks and the group receiving 3 exposures of the combined treatment per week for 12 weeks plus promotion by TPA. In contrast, the incidence of tumors is greater in the HRS/J/Anl mice given 3 exposures/week for 12 weeks plus TPA than in those given 5 exposures/week for 24 weeks without TPA. The virtual elimination by TPA promotion of the difference in tumor response between strains treated with 8-MOP and UVR suggests that much of the strain-dependent difference in epidermal tumor susceptibility to the combined 8-MOP and UVR treatment lies in the expression rather than initiation of tumors.

Previous results (*15*) suggested that exposure to 280–400 nm light at nontumorigenic fluences immediately before or after the combined 8-MOP and 365-nm irradiation shortened the time of appearance of skin tumors. However, in this experiment, it was necessary to use 3 compared with 5 exposures/week used in the 8-MOP and 365-nm exposures. To verify this suggestion, we repeated the 8-MOP plus 365 nm for 24 weeks but with 3 exposures/week; the preliminary results indicated that tumors appeared earlier with this number of weekly exposures. That the effect on tumor incidence due to the interval between fractions seen in the 24-week regime is caused by the differences in initiation events is suggested by the data in text-figure 5. The incidence of tumors is greater with 36 exposures (3/wk) than with 35 exposures (5/wk) when TPA is given to maximize the expression of the initiation events. The data for the 18 exposures (3/wk) are also shown to indicate the dose–response relationships for the 3/week regime.

DISCUSSION

The results reported here confirm the previous findings (*1, 2, 4, 5*) that photosensitization of the skin of hairless mice increases the tumorigenic effect of UVR. In all previous studies, a broad-spectrum UV light source was used, so that lesions other than the psoralen photoadducts might have influenced tumor induction. Our findings showing carcinoma induction by the combined treatment of 8-MOP plus 365-nm UVR suggest that the photoadducts induced by this treatment are related to the tumorigenesis. However, our results do not establish clearly a relationship between psoralen–DNA cross-links and tumor induction because of the different tumor incidences in the two strains exposed to regimes which, we believe, induce the same amount of DNA cross-links. This apparent lack of relationship between the psoralen–DNA cross-links and tumorigenesis may be misleading, because it appears that the strain-dependent difference in tumor incidence is largely due to the difference in the expression and not initiation events. We are further investigating whether the DNA cross-links are the causal lesions in the induction of the skin tumors in a current experiment in which the incidence of tumors is being determined as a function of the dose levels of 8-MOP and UVR. The range of doses of the combined treatment in this experiment induces a fourfold range in DNA cross-links. It will also be necessary to determine whether the monofunctional adducts influence tumorigenesis.

We believe that the time–dose relationships for the induction of tumors with psoralen and UVR are more complicated than we expected and that they raise a number of problems which will need further investigation before the underlying mechanisms can be understood.

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Interaction of Light and Chemicals in Carcinogenesis[1, 2]

Ronald E. Davies[3]

ABSTRACT—Interactions between chemicals and UV light, as they may affect the carcinogenic process, are examined in terms of the three-component system: chemical, light, and biologic target. Various two-component interactions, in addition to unique three-component interactions, are considered. Available information is incomplete concerning such possibilities as promotion by noncarcinogenic light, the contribution of acute or chronic phototoxic events to chemical or physical carcinogenesis, and the relationship between either photochemical carcinogenesis or chemically enhanced photocarcinogenesis and demonstrable phototoxic activity. Interactions such as optical absorption by, or photochemical alteration of, chemicals are considered primarily as confounding variables in experimental situations. It is argued that realities of human exposure may reduce the complexity of these problems in practical safety or regulatory considerations.—Natl Cancer Inst Monogr 50: 45–50, 1978.

That light and chemicals may interact in carcinogenic processes has been suspected since Findlay (*1*) showed that tar plus UV light was more carcinogenic than tar alone. This initial, straightforward study gave little warning of the complexities to come.

A distinction can be made among at least three broad classes of experimental studies of light, chemicals, and carcinogenesis. Although some areas overlap, the distinction is useful to separate the purposes of the studies and the types of conclusions to be drawn from them.

CLASSES OF EXPERIMENTAL STUDIES

Optical or Photochemical Properties of Chemicals

Studies of this type form a subset of a much broader area of investigation that attempts to relate objective physical and chemical parameters to biologic effects. Notable examples include studies of absorbance (*2–5*), fluorescence or phosphorescence excitation energy (*6–8*), and various photocoupling or photooxidation reactions (*9, 10*). With few exceptions, these investigations involve precise measurement of the selected chemical or physical parameter for a series of compounds (frequently, though not always, polynuclear aromatic hydrocarbons). The results are then compared with some index of the carcinogenicity of the compounds (e.g., Iball indices), often as qualitative "carcinogenic-versus-noncarcinogenic" categories. The correlations observed are generally greatest if the range of chemical compounds is restricted; no single characteristic has been observed to be correlated perfectly with carcinogenic activity, although Birks (*7*) and Morgan et al. (*8*) reported a fairly strong association with the energy level of the first excited state.

Photobiological Properties of Chemicals

The ability of compounds to sensitize some biologic property (survival, movement, etc.) to deleterious effects of light is compared by some objective (death, immobilization) or semiobjective (erythema, vesciculation) criterion. Studies of this type include those of Mottram and Doniach (*11*), Epstein (*12*), and Morgan and Warshawsky (*13*). As in the previous research, the resulting measurements are compared with some external classification of compounds as carcinogenic or noncarcinogenic. Again, correlations are best when the range of chemical species is limited.

In both categories of study, the usual stated objective is to reveal some structural or mechanistic characteristic that may be functionally related to carcinogenic activity. An implied objective is to provide some convenient means for rapid prediction of carcinogenic potential.

Light and Chemical Interactions in the Carcinogenic Process

This category, with which we will be most concerned, involves experiments in which tumors are actually produced. The focus of interest is usually some quantitative measure of carcinogenic efficacy, and the questions asked deal primarily with interactive effects rather than with the main effects of the two types of agents.

PHOTOCHEMICAL PROPERTIES: AN EXAMPLE

Before further consideration of the types of interactions that may affect the carcinogenic process, I would like to describe one example of the comparison of biologic and photochemical properties.

The two compounds shown in text-figure 1 have a number of properties in common: Both are 1) structural derivatives of benz[*a*]anthracene; 2) strongly fluorescent and highly photosensitive; and 3) potent cutaneous carcinogens. There is also considerable analogy in the way they are metabolized by rat liver homogenates. MCA is preferentially hydroxylated on one or both of the external

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Abbreviations: MCA = 3-methylcholanthrene; DMBA = 7,12-dimethylbenz[*a*]anthracene; UV-A = long-wave ultraviolet light (> 320 nm); UV-B = midrange UV (280–320 nm); UV-C = short-wave UV light (< 280 nm); 8-MOP = 8-methoxypsoralen.

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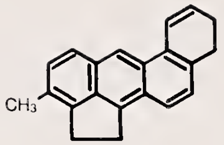
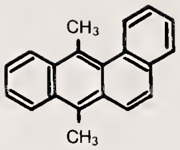
[2] Supported by Public Health Service grant CA11536 from the National Cancer Institute and grant ES00269 from the National Institute of Environmental Health Sciences.

[3] The Center for Photobiology, Skin and Cancer Hospital, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140.



MCA



DMBA

(dimethylene) bridge carbons and to some extent on the methyl group; some K-region and other epoxidation and formation of a glutathione conjugate also occur (*14*). With DMBA, the primary action of rat liver is monohydroxylation at either of the two methyl groups; some production of dihydrodihydroxy derivatives is thought possible specifically on the end rings (3–4, 8–9, 10–11) but not at the K-region (*15*). The primary reactions (methylene or methyl hydroxylation) may have no effect or may reduce the carcinogenic potency compared with the parent compounds.

Irradiation of MCA solutions produces a mixture of products similar to that resulting from the metabolic reactions described above [(*16*); Logani MK, Austin WA: Unpublished observations). Although this was interpreted by Dao et al. (*16*) as a solvent-dependent (dimethyl sulfoxide) photooxidation, essentially the same products are formed in a variety of solvents (hexane, benzene, carbon disulfide, CCl_4 etc.) (Logani MK, Austin WA: Unpublished observations). In sharp contrast, photooxidation of DMBA is a two-stage reaction proceeding through 7,12-epidioxide formation and involving only the substituted ring. Side-chain reactions are secondary and involve displacement (e.g., quinone or substituted anthrone formation or methoxy formation). The major stable product is a substituted acetal (*17*). None of these photoproducts exhibits detectable carcinogenic potency.

Obviously, irradiation in solution is not a direct analog of either biologic reactions or even in vivo irradiation. The point I wish to make is that these 2 compounds, with a great deal in common with respect to structure and biologic activity, are unalike photochemically, and any attempt to predict their biologic properties from their photochemical reactions would be highly misleading.

TERMINOLOGY OF INTERACTIVE PROCESSES

To return to the question of light plus chemical interactions in the carcinogenic process, it is necessary to define some concepts, which are either not clearly defined or for which the terms used have different meanings to different people.

"Carcinogenesis" refers to a response by a multicellular biologic system to a physical, chemical, or biologic stimulus; the agent capable of eliciting this response is referred to as a "carcinogen." The type of agent involved is generally specified in a qualifier; e.g., "chemical carcinogenesis," "photocarcinogenesis," etc.

By analogy with this terminology, the expression "photochemical carcinogenesis" refers to production of tumors in response to a combination of light and chemical. This usage implies that the response is uniquely associated with the combination, and thus the two must be present simultaneously. Conceptually, the process is independent of the individual carcinogenic activity of light or chemical; practically, these independent activities provide serious problems in the identification of photochemical carcinogenesis as a contributory process.

"Initiation" and "promotion" are complementary terms that describe a particular type of interactive process. An initiator is a carcinogen which, under the conditions of a particular experiment, may be an inadequate stimulus for the production of overt tumors; under more severe conditions of dosage, application frequency, or by a different route of administration, it would be expected to produce tumors. In contrast, a promoter is considered to be incapable of eliciting tumors under any conditions. However, after the action of an initiator, a promoter will stimulate the appearance and growth of presumptive tumors by a process which is, by definition, more than additive. This phenomenon is generally explained in terms of two (or more) distinguishable stages in the carcinogenic process. Some inevitable ambiguity results from the difficulty in defining a compound as absolutely noncarcinogenic (and thus qualified to be a promoter). It is also conceivable that a compound could have such low carcinogenic activity as to be undetectable by any direct assay, and yet sufficient to initiate a promotable response; such a compound would appear to violate the concept that an initiator is a "complete carcinogen." I am not aware of any examples of such an anomaly.

A related but distinct concept is that of "co-carcinogenesis." Although sometimes used interchangeably with the initiation–promotion concept, the term will be used here to refer specifically to the combined activity of two effective carcinogens, though either or both may be present at levels too low to produce an independent response. It is possible that no fundamental difference exists between co-carcinogenesis and initiation–promotion carcinogenesis with respect to their biologic action, but they present different problems of experimental design and evaluation. Both initiation–promotion and co-carcinogenesis have been observed in light plus chemical carcinogenesis.

I will use the term "chemically enhanced photocarcinogenesis" to refer to the situation in which a photochemical process serves as a promoter and carcinogenic light is the initiator. The difference between chemically enhanced photocarcinogenesis and chemical (e.g., Croton oil) promotion of photocarcinogenesis lies in the fact that the former involves photochemical (rather than chemical) promotion. Of course, if the photochemical process alone can induce cancer, the result would be a type of co-carcinogenesis involving chemical and photochemical carcinogens. Distinction among the various possibilities can impose methodologic problems and may be of limited significance from the protection or regulatory viewpoint. Nevertheless, the concepts are important because under real conditions one effective carcinogen, light, is inevitably present, though possibly at ineffective levels. Thus promotion and co-carcinogenesis, whether chemical or photochemical, are potentially important components of UV carcinogenesis in human populations.

TYPES OF INTERACTIVE PROCESSES

Some (but not all) component wavelengths of light, and some (but not all) chemicals are capable of acting as carcinogens. Thus the interaction of light and chemicals in carcinogenesis may involve up to two carcinogen-plus-target systems, other interactions between any two members of a three-component system, or any unique three-component interactions (including carcinogenesis). As we will see, the special situation of UV photocarcinogenesis may reduce the practical importance of some difficult mechanistic distinctions.

The system we must consider consists of three components; light, chemical, and biologic target. The interactions that could result will vary depending on whether the light or the chemical (or both) is (are) independently carcinogenic (see table 1). We will consider the general types of possible interactions, noting when appropriate the expected direction of changes and known examples.

Two-Component Interactions

Light and Chemical

Optical/physical:—The chemical modifies the spectrum of input light prior to interaction with the biologic system. Extreme examples include attenuation of the solar UV spectrum by stratospheric ozone, and absorptive modification of biologically effective input radiation by superficial skin layers (e.g., stratum corneum). The major group of materials for which this type of interaction is of deliberate significance is the topical sunscreens. However, it can also be a confounding factor in other interactions as discussed below. The expected direction of effect is reduced biologic activity of a given light input.

A second possible type of light spectrum modification could result from excitation and emission of luminescence (fluorescence or phosphorescence). Since 1) the direction of such a shift is (virtually) always to longer wavelengths; 2) in light sources of practical importance, the shortest wavelengths emitted have the greatest biologic activity; and 3) luminescence emission is omnidirectional, this type of spectrum modification would probably be indistinguishable from absorptive attenuation in practice.

TABLE 1.—*Interaction among light, chemicals, and biologic targets* [a]

Light	Chemical: Noncarcinogenic	Chemical: Carcinogenic
Noncarcinogenic	Phototoxicity Photochemical carcinogenesis Photochemistry Optical filtration	Photochemistry Phototoxicity Promotion Host effects Optical filtration
Carcinogenic	Optical filtration Promotion Photochemical cocarcinogenesis Host effects Chemically enhanced photocarcinogenesis Phototoxicity Photochemistry	Photochemistry Cocarcinogenesis Optical filtration Chemically enhanced photocarcinogenesis Photoxicity

[a] Underlined categories include known examples, and the remainder are not demonstrated or are of uncertain significance.

Photochemical.—Light directly alters the chemical to one or more products with different biologic activity. Known examples involve reduction of carcinogenic activity (e.g., photooxidation of DMBA or of MCA), but this is not necessarily the direction of change. One reported photoproduct of mirex is Kepone (*18*), which is carcinogenic (*19*), but whether it is more or less so than mirex is not known.

In addition to its potential importance in the destruction (or production) of environmental carcinogens, this class of interactions is of significance as a frequently unrecognized, potential confounding variable in carcinogenesis and photocarcinogenesis assays.

Chemical and Target

Promotion.—The chemical enhances the survival or growth of tumors elicited (initiated) by carcinogenic light. This effect has been demonstrated with Croton oil (*20*). In the usual initiation–promotion study, the two stages are separated in time, so ambiguity is minimized. When promotion in the presence of chronic or recurrent light treatment is to be studied, care must be taken to distinguish it from chemically enhanced photocarcinogenesis.

Host modification.—The responsiveness of the target to the carcinogenic light stimulus is altered. Examples include suppression of immunologic activity by antilymphocytic globulin and (presumably) tumoristatic or tumoricidal activity of 6-mercaptopurine (*21*).

A trivial example would be depilation of a haired target (or other removal of natural filters) increasing the effective dose of light on the target. Conversely, a chronic irritant might thicken stratum corneum or destroy actually or potentially mutated cells, reducing the apparent susceptibility of the target to light carcinogenesis. A more convoluted example involves the apparent protective effect of antioxidants; one proposed mechanism involves inhibition of photooxidation of cholesterol to a carcinogen or carcinogen precursor (*22*).

Initiation or co-carcinogenesis.—The chemical is an independently effective carcinogen. Its effect may not be contingent on that of light, or it may be additive to any carcinogenic influence of the light, or the two may be interactive. It could also act as the initiator of a carcinogenic stimulus to be promoted by light; however, the promotional characteristics of noncarcinogenic light have not been definitely established.

Light and Target

Host modification.—The responsiveness of the target to a carcinogenic chemical is modified. There are no clear examples of this type of interaction. The influence of light on the immunologic response to UV-induced or transplantable tumors (*23*) may have an analog in chemically induced tumors, although the latter generally have low antigenic activity. Some have suggested that light-influenced pineal activity could affect susceptibility of pigmented tissues to carcinogens.

Promotion.—Noncarcinogenic light enhances the growth or survival of chemically induced tumors. Despite indications that UV-A light can potentiate some type of damage (usually induced by UV-B), no clear evidence is available that UV-A can act as a promoter. The area remains speculative.

Three-Component Interactions

Light, Chemical, and Target

Acute phototoxicity.—The combined action of light and chemical produces severe trauma in the target. Since many cells may be destroyed in this process, potential tumors induced by a carcinogen (chemical, physical, or photochemical) may also be destroyed. This may be an important process in many of the negative responses described in photochemical carcinogenesis studies (*24–26*). On the other hand, it is known that single, acute UV-B exposures, accompanied by severe trauma, can lead to tumor production (*27*); phototoxic trauma may have similar effects.

Photochemical carcinogenesis.—The combination of light and chemical exhibits carcinogenic properties not exhibited by either alone. The one clearly documented example is 8-MOP, which has produced this effect topically and systemically (*28, 29*). Although 8-MOP is phototoxic, the carcinogenic process can occur in the absence of observable acute phototoxicity. Not all phototoxic compounds can produce photochemical carcinogenesis (*30*). However, few studies have excluded possible direct effects of UV-B to eliminate the complications of either promotion or chemically enhanced photocarcinogenesis.

Co-carcinogenesis.—Both chemical and light are independently active. Little quantitative evidence is available to distinguish between additivity or syngergism. However, such interactions may well be synergistic. Interestingly, the tumors produced in mouse skin in response to chemical (aromatic hydrocarbon) carcinogens have qualitatively different morphology from those induced by UV. A combination of the two produces large numbers of tumors, exclusively of the chemically induced type (Forbes PD: Personal communication).

Chemically enhanced photocarcinogenesis.—The response to carcinogenic light is enhanced by a photochemical process analogous to promotion. This process has not been clearly demonstrated: In the presence of UV-B and UV-A, 8-MOP increases the yield of tumors, but since the combination of 8-MOP plus UV-A is carcinogenic, this is the photobiologic analog of co-carcinogenesis rather than promotion. In contrast, anthracene appears to produce no such photochemical promotion despite its known phototoxic activity (*30*). In most other studies of this type, carcinogenic chemicals have been used, so the relevance of this type of reaction is uncertain. Bingham and Falk (*31*) reported a response to optical brighteners that apparently is of this type. In their work, the carcinogenic light was UV-C (shortwave). Other studies with the same brighteners failed to confirm this response in the presence of either UV-B or UV-C as an initiator (*32–34*).

PROBLEMS IN EVALUATION OF INTERACTIONS

In addition to the basic problems of distinguishing between the various possible types of interaction, the investigator is faced with the real problem that two or more types of interaction will frequently (usually) occur simultaneously. This raises the question of the significance of the combined effects likely to be seen in the real world, and also the problem of how best to detect and measure them. Experimental designs may be intricate and must be evaluated in terms of the ambiguity of any possible outcome.

In the evaluation of human hazard, contrastingly, several simplifying circumstances and assumptions reduce the complexity of the problem. For example, co-carcinogenesis implies that the chemical agent is a carcinogen, and can be detected and regulated as such. Acute phototoxicity is an immediate and specific problem, and the necessary measures to prevent its occurrence would likewise prevent any resulting carcinogenic hazard. In a contrasting sense, UV light is an effective and universally present carcinogen, so the distinction between chemical promotion and co-carcinogenesis or between photochemical enhancement and photochemical carcinogenesis may not need to be rigorously established. In another context, Roe and Tucker (*35*) have argued against "a definition of carcinogenicity which embraces co-carcinogenicity and non-specific enhancement . . . ," but their point was that the test conditions should be realistic. Realism involves recognizing the presence of carcinogenic light in the normal environment. A compound with promotional or photoenhancing properties could function as an apparent carcinogen in human populations and be detected as such in epidemiologic studies, but it would be ineffective in standard carcinogenesis assays.

For the above reasons, practical evaluation of light plus chemical hazards can probably be simplified to two situations: Chemically enhanced photocarcinogenesis, in which one evaluates the effect of a chemical on carcinogenic natural (broad-spectrum) light, and photochemical carcinogenesis, in which the UV-B portion of the light spectrum can be eliminated and the total light dose correspondingly increased. In each instance, the use of light and chemical should be prolonged and, so far as practical, similar to real exposure conditions. Whereas positive results of such trials would not define unambiguously the mechanism of interaction, they would indicate the existence of a potential hazard.

A special problem in the design of such trials should be noted. Bioassay procedures, including carcinogenesis assays, incorporate the assumption that a positive association exists between dose and response. A typical approach in carcinogenesis assays involves use of the maximum tolerated dose (as judged by mortality or morbidity responses in subchronic trials) and one or more lower doses, with the expectation that in the absence of other deleterious effects, the largest dose will produce the largest response.

This assumption cannot be transferred uncritically to assays for photochemical carcinogenesis or chemically enhanced photocarcinogenesis. In each instance, the possibility of interaction between light and chemical (or chemical-containing complex) must be assumed; for such interaction to occur, some absorption of light is required. If the chemical itself absorbs active wavelengths (whether photocarcinogenic or activators of photochemical carcinogenesis), the amount of light reaching the site of action may be reduced in direct proportion to the dose of topically applied chemical. In a photochemical carcinogenesis assay, the result could be the equivalent of self-screening in dense solutions; in one for chemically enhanced photocarcinogenesis, a pure sunscreening action could result. In either case, although a positive dose–response relationship might be expected at low doses of chemical, the relationship could fail or even be reversed at higher doses. For this reason, care and judgment are required in experimental design, and statistical evaluation should not include an automatic dose–response assumption. Although this problem is most acute in topical application of test material, it should not be ignored when other routes of administration are used; since such studies assume that some of the test substance will reach the cutaneous system, systemic sunscreening remains a possibility.

SUMMARY

UV light can initiate or alter carcinogenic processes, interact with or modify chemical carcinogens, and participate in photochemically mediated biologic reactions. In the laboratory, it may be a systematic or an uncontrolled (or even unrecognized) variable in carcinogenesis studies. More generally, it is a significant and difficult factor in the complex problem of environmental carcinogenesis.

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Discussion: Experimental Ultraviolet Radiation Carcinogenesis[1, 2]

F. Urbach: I would like to take the liberty as Chairman to ask Professor Latarjet to start the discussion.

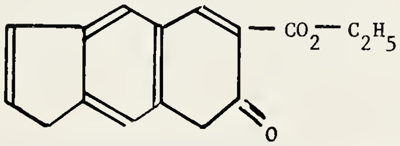
R. Latarjet: I am extracting a short paragraph from my paper, because it seems to me that it fits in much better with the present session, in particular with the discussion of Dr. Fry's paper.

At the Institut du Radium, we have been studying various psoralens to determine their lethality and mutagenicity in bacteria and in yeasts, and also their carcinogenicity in mice. The furocoumarin is obviously associated with UV-A.

We confirmed that the bifunctional psoralens are potent carcinogens, and the only thing I can add in this respect is that by far the most potent among those we have studied is 8-methylpsoralen, which is much more active than the 8-methoxypsoralen that Dr. Fry is using.

All these compounds are bifunctional; that is, they can produce, as Dr. Fry said, monoadducts and cross-links.

To try to reduce the carcinogenic activity, in view of potential widespread therapeutic applications, we sought to block chemically one of the two active groups involved in the production of cross-links, and we prepared a series of monofunctional compounds. The most convenient one, with regard to solubility and many other characteristics, is the 3-carbethoxypsoralen illustrated below. This compound produces monoadducts only, and we found that it does not produce tumors in mice.



The obvious step was initiated last week, when we gave the compound to several dermatologists to try on patients with psoriasis.

Urbach: I think this is an extremely important observation, particularly in view of the questions that Dr. Fry has raised. It seems that we already have a partial answer, at least with one compound that only forms monoadducts to DNA.

Dr. Fry, do you want to make any comments concerning this?

M. Fry: I should have read Dr. Latarjet's abstract before I made my remarks about the future experiments with furocoumarins. How do the phototoxic effects and erythemal response differ from those with 8-methoxypsoralen?

Latarjet: The skin is remarkably undamaged with this compound, although it is active in the bacterial tests.

K. Smith: Dr. Fry, you attempted to correlate the production of cancer and the initial production of lesions. However, in all of radiation biology and photobiology, it has been demonstrated well that survival and other parameters do not correlate with the initial yield of radiation damage. The residual damage left after repair (unrepaired or incorrectly repaired) presumably causes the cancer. I wonder if you have plans to study (or already have done so) the amount of cross-links remaining at various times after the treatment to see if any strain differences were there?

Fry: We have not, but this is a good point. One would like to determine the residual amounts of cross-links in cells after the exposure regimes. That is difficult to do, but perhaps we should put that up higher on our priority list.

J. van der Leun: I would like to comment on the paper by Dr. Forbes. Dose has always been a fundamental factor in skin photobiology. We speak about the relationship of dose and effect. We speak about action spectrum, defined as the reciprocal of the dose required for a certain effect in the skin, plotted as a function of wavelength. This all presupposes that dose is an adequate description of the stimulus given. That in itself requires that "reciprocity" holds; it is only the product of time and irradiance that matters for the effect.

You have shown us experimental results that suggest reciprocity does not hold for carcinogenesis, and this seems to imply, for instance, that "the" action spectrum of UV carcinogenesis is not only unknown but does not even exist. Instead, we will at best have a set of sensitivity curves, each valid for specified experimental conditions.

Another point is that your experimental results seem to indicate an increasing carcinogenic effectiveness toward the shorter wavelengths, below 300 nm. That is a most important point with regard to the possible effects of a depletion of the ozone layer.

At the start of the discussions on the ozone layer, Dr. James McDonald made a good contribution. He pointed to the intriguing possibility of using the epidemiologic data to determine the most effective wavelengths in carcinogenesis by sunlight in human skin. The idea was that the more the frequency of carcinoma depended on the amount of ozone, the shorter the wavelengths involved should be. This line was not followed by most investigators because they preferred to work on the presupposition that the action spectrum for carcinogenesis was the same as that for erythema, and among these were powerful committees. Dr. Farrington Daniels, Jr. and I pursued Dr. McDonald's line and, after applying some corrections and refinements, we came to the conclusion that the epidemiologic data pointed to a peak effectiveness of

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Abbreviations: UV-A = 320–400 nm; UV-B = 280–320 nm; UV-C = 200–280 nm; UVR = UV radiation.

[1] Conducted at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] *Address reprint requests* for all "Discussions" to Dr. Margaret L. Kripke, Cancer Biology Program, Frederick Cancer Research Program, P.O. Box B, Frederick, Maryland 21701.

carcinogenesis by UV radiation in human skin somewhere near 270 nm.[3] This conclusion seems to be well in line with the experimental results shown now.

P. Forbes: Just one comment on that latter point. It appears increasingly clear to us that the shorter wavelengths are more effective than we anticipated, based on one of the action spectra for erythema. I think we need to emphasize continually that there are a number of action spectra, depending on how we define erythema, and one may be significantly better than another for predicting other effects.

One of the points that I find a bit difficult to reconcile is Dr. Freeman's observation that the 290-nm radiation is apparently so ineffective in producing cancer; we have no idea whether a turn-around point is somewhere in that region, but there certainly does seem to be greater effectiveness below 300 nm than we anticipated.

With respect to the first comment on experiments which only provide information about those specific conditions, that is inevitably where one must start. One hopes to generalize from specific experiments at some point. I believe that by making finer and finer dissections of the variables, we will find data that help us to identify interactions.

I. Willis: I have two questions. The first one is directed to Dr. Forbes. As you know, our photoaugmentation or photoaddition phenomenon essentially shows that UV-A enhances the erythemal effects of UV-B. Your experiments with combinations of UV-A and UV-B versus UV-B alone did not reveal augmentation for carcinogenesis. Also, our results show that histologic damage is greater with combined UV-A and UV-B and, therefore, I would expect carcinogenesis to be augmented. Yet in your study it is just the opposite. Were any histologic studies included in your experiments to see whether, in the early phase of the irradiations, the damaging effects with UV-B combined with UV-A were decreased? In addition, the results of my experiments differ from yours, in that I see an enhancement of carcinogenesis by combining UV-A with UV-B.

The second question is to Dr. Epstein. You mentioned in your discussion that retinoic acid enhances UV carcinogenesis and, as you know, recent claims state that retinoic acid can help eliminate prematurely aged skin or reverse some of the changes. Am I correct in my interpretation of your carcinogenesis experiment and, if so, should a revised recommendation be given to people who have damaged skin and are now using retinoic acid to treat it?

Forbes: We could not differentiate between the treated groups histologically. That is only a qualitative observation. No, we did not see an enhancement that we could detect from histology. I would like to add one comment on combination experiments. If an experiment is designed to test the effect of added UV-A, all animals should be exposed to the same primary (UV-B) source. As soon as you switch UV-B sources, you get into trouble. As far as I am concerned, the question regarding UV-A effects is certainly not closed. Information is still to be obtained, but it needs to be defined carefully in terms of the spectra used.

Urbach: Incidentally, one must also consider the quantity or the relative distribution of UV-B and UV-A. I am not familiar with your experiments, Dr. Willis, but I would not be surprised if the relative amounts of UV-A might differ from the amounts that Dr. Forbes has used. Quantity may well influence the results, as suggested in Dr. Forbes' slide. When a significant amount of UV-B was given first, the UV-A effect was negligible. When a small amount was given and then followed by UV-A, the interaction became more striking. These kinds of interactions are probably always going to be related to some quantitative considerations, but it will take a lot of work to define these.

J. Epstein: I would like to add to the answer to Dr. Willis' question. You used the same amount of the UV-A plus the UV-B that would induce the augmented damage. Is that right?

Willis: That is correct.

Epstein: As I understand it, Dr. Forbes used a much smaller amount of UV-A. Therefore, he most likely would not see the enhancing effect that you obtained. It sounds like a dose-related phenomenon.

The answer to Dr. Willis' question regarding retinoic acid is a thorny one. Actually, a fair number of reports show that retinoic acid and some of the other analogs will inhibit carcinogenesis under certain circumstances. Nonetheless, retinoic acid, which was used in a large irritating amount, was not carcinogenic but did promote tumor formation in our system. We are now in the process of using much lower concentrations which are not irritating. So perhaps in a year, I will be able to tell you whether the lower concentrations of retinoic acid will also act as promoter.

Forbes: I should have indicated that one point of the combination light source experiment was to keep the UV-A in the same proportion to UV-B because it would be in a specified solar spectrum, and the conclusion can only apply to that combination. Thus our conclusion is that, when UV-A and UV-B are in such proportion as they would be in sunlight, UV-A did not enhance photocarcinogenesis.

J. Chan: I would like to comment on Dr. Freeman's finding about a parallel between his erythema action spectrum and the carcinogenic action spectrum.

We did some studies during which we fed animals with a diet supplemented by 4 antioxidants and found inhibition of UV-induced tumor formation in hairless mice. We also did some studies in which the animals, instead of a mixture of 4 antioxidants, received a diet that contained only 1 (butylated hydroxytoluene) of the 4 antioxidants, and we found that the supplemented diet was the only one that protected against UV radiation, and the other 3 did not.

We also did some experiments to determine the ability of individual antioxidants to protect against UV-induced erythema, and we found that the butylated hydroxytoluene-supplemented diet was the only one that gave any protection against erythema.

Our experimental results seem to support what Dr. Freeman found: a parallel between the erythema action and the carcinogenic action spectrums.

R. G. Freeman: Dr. Chan and I discussed this earlier. I find it interesting, and I am, of course, grateful for anything that might confirm what we found.

D. Krizek: Dr. Forbes, I found your report stimulating, but I was concerned about whether you had, in fact, filtered the FS-40 fluorescent lamps in your studies to remove the UV-C radiation (200–280 nm) emitted by these lamps.

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[3] Daniels F Jr, van der Leun J: Impacts of climatic changes on the biosphere. *In* Ultraviolet Radiation Effects (Climatic Impact Assessment Program Monogr 5, Part 1). Springfield, Va.: Natl Tech Inform Serv, 1975, pp 7.105–7.124.

Forbes: The FS lamps were not filtered. Depending on how you determine their spectrum, their output extends below 280 nm.

Krizek: I would like to suggest to those researchers who are using FS-40 lamps as a UV-B source to try filtering them with cellulose acetate, or other suitable filters, to see if they might not, in fact, get different effects than when UV-C radiation is present. Certainly in the plant world, we have obtained dramatically different responses, depending on whether FS-40 lamps were filtered appropriately. Although the published curves on FS-40 lamps do not show a large amount of UVR below about 280 nm, a substantial amount is there (including 254-nm radiation), which can have a dramatic effect on biologic organisms. I would like to caution investigators who use these lamps.

Forbes: Can you give some estimate of the amount of what you are calling UV-C in these lamps?

Krizek: I cannot give you the numbers offhand but would be happy to obtain them for you since we have taken spectroradiometric measurements on these lamps at the Department of Agriculture, Beltsville, Maryland. In total percentage, the UV-C component (including 254-nm radiation) is small. In biologic effectiveness of the UV-C component present, however, this percentage may be appreciable.

Urbach: Please remember that the Kodacel filter cuts off sharply at about 295 nm or thereabouts, and certainly the fluorescent sunlamps have a significant contribution below 295 nm. You are taking out an extremely potent, biologic component of these lamps. We seem to have a problem with the definition of UV-C.

E. Scott: I want to go back to something that was discussed in the early talks, when Dr. Urbach and Dr. Blum both mentioned that the data of melanoma incidence as a function of latitude or of UV flux seemed more uncertain than nonmelanoma and, therefore, it may have less association. I want to suggest that this is largely a statistical artifact. Because melanoma is a much rarer disease than nonmelanoma, the number of cases is small, and so you are bound to get much more fluctuation on the plot. On the other hand, if you take, instead, the melanoma mortalities, for which we have much more data collected for a longer period, you find a good association.

Urbach: That certainly is a possibility. However, I should also point out that the melanoma data that Dr. Blum showed is based on nine places in the country, whereas the data on squamous carcinoma was only based on four. The problem of numbers is a serious one. As we get more data from the Surveillance, Epidemiology and End Results surveys and others, we will be able to come closer to some better relationships.

D. Roth: With respect to this skewing of the melanoma data that was mentioned by Dr. Scott, we should remember that the distribution of melanomas on the body does not correspond to the sun-exposed areas. The highest incidence is on the trunk and the legs. It may be that those that arise in sun-exposed locations have a direct relationship to light, whereas those that arise on the trunk may have an indirect relationship through some chemical mediator or other factor. Thus you would expect to get a skewing of the incidence of melanoma, because of an indirect factor that is still undefined.

Urbach: The main point in the slide shown by Dr. Blum was that he used the identical criteria for plotting a form of dose–response relationship, if you like, by taking data from UV measurements at some latitudes, measuring the incidence at various places, and plotting it in relation to the dose.

If one then does this in the same fashion for basal cell and squamous cell carcinomas versus melanomas, the former show a nice straight-line relationship to dose and in the latter case of melanoma, it is far from that. This simply means that by using the same analytical technique for comparing two sets of data, one comes up with different answers. However, Dr. Scott's point is eminently well taken. Because few patients have melanoma, the variability may be much greater.

Dr. Magnus points out (in his paper) the biggest problem in trying to set up dose relationships of UVR to human skin cancer. We are now capable of measuring reasonably well the total UV-B dose (as multiplied by an action spectrum) at ground level in various places. However, we do not have the foggiest notion of what percentage of that dose the patient who develops the skin cancer received. An hour of exposure at noon is completely different from an hour in the afternoon. Until we have some sort of personal dosimeter to give us some idea of the fraction of the daily dose received by the person-at-risk, all dose–response calculations will be inaccurate. At the present time, the dose–response relationships are estimated in relation to either total daily, monthly, or annual UV doses or some arbitrary fraction thereof. At this time, we have no way of estimating intelligently what is the actual dose received by the patient. Dr. Lee wanted to comment on that presumptuous statement. I stand by it, though; under the present circumstances, I do not know how one can calculate UV dose in man except in the vaguest possible way.

J. Lee: I am sorry, Dr. Urbach, I was not going to comment on your point. I want to return to the melanoma discussion. The latitude relationship is just as tight for melanoma as for the squamous cell carcinomas. Also, a concentration of melanomas is on the exposed surface of the skin if you calculate it per unit area of skin. It is not as big as that for the squamous cell and basal cell carcinomas, which must be enormous, but it is certainly there. The problem that I think is producing the differences, to which Dr. Blum also refers, is that any estimate that you make of the rate constant, linking age to melanoma, is being perverted by two factors: One is the rapid rise in the incidence of melanoma, and the other is the improvement in treatment in recent years. I suspect that by putting in a rate constant of about 4 for a melanoma (which I think is what the real one is) instead of the rate constant of about 2, you should get cross-sectional data, which are about all we have, except for mortality, and you might find that the whole thing resolves itself.

Urbach: The business of relating tumor incidence to area of skin is, as far as I am concerned, totally specious. If you look at squamous cell or basal carcinoma per area of skin, you will not find any on the belly, on the buttocks, or over a large part of the body, simply because these skin areas are not exposed to UVR. You saw that slide showing the geometry of body exposure. Even if you did not wear any clothes, if you went completely naked, you would have a great deal of difficulty getting sufficient amounts, or anything near the same amount of UV light on your pubic area as you will get on your nose. It is just a geometric impossibility. Therefore, this famous statement that I keep hearing over and over again, that one should evaluate melanomas "per unit area," is totally inappropriate. This does not have to do with biology, but with geometry, since light passes in straight lines and human beings who develop cancer tend to be exposed to UV while upright. You know, if everybody were lying flat on his back from daybreak to sunset, on a spit, then the "tumor per unit of exposed area" concept would be an appropriate statement, but people do not work that way. So I hope that this business of tumors per exposed square millimeter of skin will get laid to rest once and for all. It is just simply not so. I am sorry, Dr. Lee, but my sentiments about this horrible fallacy are strong.

Epstein: The difference in the relationship of latitude to melanoma versus nonmelanoma skin cancer may relate to changes in the host response. The possibility that we are dealing with other host changes via UVR in melanoma, I think, has to be considered.

Urbach: Oh, absolutely. I did not mean to imply that latitude was unrelated to melanoma.

Scott: Could I pick this up for just a moment? Without contradicting what Dr. Urbach says, we have to take into account that, in the middle latitudes of the United States, about half the intensity of UV is coming from scattered light; so it is not only the top of a person that is irradiated. Also, with regard to protection from clothing, one must realize that clothes are not totally protective. Approximately 50% of the UV light is transmitted through a man's shirt. I believe you have to consider something else, which is shown strikingly from the study of the other Dr. Magnus in Norway, confirmed by Dr. Lee, and which we see now from recent data in the United States: The sites of melanoma are highly dependent on age. Younger people are getting many more on the back, etc., than are older persons; many more occur on young women's legs than in older persons; and that this, then, is in a way a change of life-style. When my father taught me to swim, and this, of course, dates me, he wore a bathing suit that had half sleeves on it and trunks that looked like short pants, whereas the man next door to us goes out to swim in absolutely nothing; you can see that the situation has really changed.

Urbach: That, plus the UV-transmitting bikinis, will give the dermatologists more work to do in the next generation.

T. Coohill: I have a question for either Dr. Forbes or Dr. Urbach that is in line with Dr. Urbach's asking for more epidemiologic data. The question is: Has anyone tried to modify the latitude data for skin cancer incidence by adding the effect of ethnic origins among the populations of various cities? For example, Boston has a large Irish population; Houston and Dallas have a large Mexican population. I notice that the data for New Orleans shown by Dr. Forbes were lower than expected. Could this be due to the large Cajun population in that city?

Urbach: Perhaps I should answer that. The one place where that has been done well is in Australia: Dr. Silverstone, at the conference held here in 1964, showed some excellent data for Australia. Since originally all the white immigrants to Australia came from one place, they have immigration records going back to the 1700's. Most of the original settlers were of Celtic origin, and about 100 years ago, there was a large influx of Italians. On the western seaboard of Queensland, Silverstone found a striking latitude gradient for incidence of skin cancer among those of Celtic and northern English origin, and a lesser gradient for that of people identically exposed who were of Italian origin. A little of that kind of data is available but not a great deal.

The data that Dr. Forbes showed came from the First National Cancer Survey. Although the data are not the most conclusive, it was impossible to make a more careful study; however, all kinds of factors entered into the results. One can see the huge difference between Philadelphia and Denver, for instance. Philadelphia is well below the expected and Denver is way above. A good deal of this, of course, is simply due to environmental conditions. Certainly the people who had cancer by 1948 and who had grown up in Philadelphia from the turn of the century or into the 1920's had relatively little UV exposure. In the winter, they had virtually none and in the summer probably only a little. However, the people who lived in Denver at that time were exposed a great deal, not only in the summer but also in the winter when the UV snow reflection is 100%. In addition, Denver is 1 mile above sea level; it prides itself as being the "mile high" city, whereas Philadelphia is at sea level. Automatically, therefore, Denver would have had 15% more UVR, simply because of having less air due to its altitude.

I think these differences that you saw, although they are in part genetic, probably have more to do simply with the amount of radiation that it is possible for people to receive. I point out again that some complicated factors enter into skin carcinogenesis from the point of view of habits, exposure, clothing (as Dr. Scott pointed out), the amount of UVR reaching the area, snow reflection, wind, and "who knows what." Therefore, it is extremely difficult to interpret some of these data.

Lee: I have considerable reservations about trying to psyche up correlation diagrams, but my statistical colleagues, Dr. van Belle and Dr. Fisher, played with this one. They simply split the United States into those states with a seacoast and those without and recorded the death rates from malignant melanoma in males. The rates are consistently higher across the latitude range for states that have a seacoast compared with those that do not. There is no substitute for the proper clinical epidemiologic studies that have to be done in the future, but this does show the kind of opportunity, of which the speaker a moment ago spoke, for doing some work to improve the gross relationships.

Scott: We did the same thing by county, instead of by states, and got tighter relationships.

Urbach: I should also point out to you that one can find explanations for good relationships that are not immediately apparent. Dr. Gordon, in Australia, did a study relating skin cancer to the wearing of hats and found that the people who wore hats had much more skin cancer (highly significant) than the ones who did not. At first glance this seemed peculiar, until he went and interviewed some of these people who wore hats and found out that the only people who wore hats were the ones who were highly susceptible to sunburn; hence the correlation. The wearing of hats was related to the ease of sunburning, which, of course, is related secondarily to skin cancer.

SESSION II

Characteristics of Ultraviolet-Induced Tumors

Chairman: M. G. Hanna, Jr.

Co-Chairman: Joseph DiPaolo

Life History and Histopathology of Ultraviolet Light-Induced Skin Tumors[1, 2]

Frej Stenbäck[3, 4]

ABSTRACT—The life history and histopathology of UV light-induced skin tumors were studied in NMR rats, outbred female Swiss mice, and Syrian golden hamsters. High intensity UV light of medium wavelengths produced hyperplasia and papillomas, as well as a dysplastic, intermediary solar keratosis-like stage, with distinct cellular atypia leading to several types of squamous cell carcinomas. High doses of UV irradiation of short duration caused scars, which developed into fibromas and fibrosarcomas composed of "light" and "dark" cells. Carcinomas with neoplastic squamous and fibrous components were uncommon; however, collision tumors with two components were occasionally seen. Angiomas and angiosarcomas with a proliferating endothelial structure were observed, but adnexal tumors, with follicular or sebaceous differentiation, and basal cell carcinomas were infrequent. Pigment cell tumors were found only rarely. The number of tumors and tumor-bearing animals at different stages of the experiment were also studied. Tumors were compared with lesions induced by chemical carcinogens in different systems. UV carcinogenesis was characterized by many tumor-bearing animals, but with a low total tumor count and a high mortality, thereby decreasing the number of animals-at-risk. The tumor types, their progression from one type to another, and the distribution of certain biologic characteristics were also analyzed. We concluded that UV irradiation is an effective tumor inducer in animal skin, and the type of tumor, its behavior, and location depend on the experimental conditions.—Natl Cancer Inst Monogr 50: 57–70, 1978.

The neoplastic response to UV irradiation, the type of tumors induced, and their morphology and biology are determined by several factors. Most important are the animal strain and species used, the type of light source, and treatment intensity and duration (*1–3*). When compared with repeated carcinogen or initiation–promotion treatment, as a tumor-inducing agent UV irradiation was significantly less effective and produced fewer tumors in all the species studied (*4, 5*). The tumors found in UV irradiation experiments are also different, compared with carcinogen-induced lesions. Carcinogen treatment induced a high incidence of epithelial and dermal tumors in mice, the adnexal type in rats, and pigmented tumors in Syrian golden hamsters (*4, 6*). These variations may be due to anatomic characteristics of the different species, penetration of UV irradiation, and the widely varying sensitivity of different tissues to carcinogen treatment (*5, 7, 8*).

The skin offers an excellent opportunity to study biologic alterations without interfering with the carcinogenesis process. This model makes it possible to observe initial alterations, the process leading to tumor formation, and the progression to death.

In the present study, we were concerned with the immediate and long-term effects of UV irradiation of different wavelengths on the skin of several animal species and strains and analyses of the results by histologic, histochemical, and ultrastructural methods. The purpose was to analyze the biologic effects of UV irradiation on animal tissues, the factors determining observed alterations, and their interrelationship and significance for tumor formation. We also wished to develop a classification system for these tumors. This report includes an analysis of the number of tumor-bearing animals and tumors at different experimental stages, their relationship to survival, tumor regression and progression, and studies on tumor behavior, the relationship between different types, time of appearance, growth rate, infiltration, and differentiation. The results were compared with those obtained from chemical carcinogenesis experiments and from initiation–promotion studies. The purpose was to determine the characteristics of neoplastic induction and progression in relation to inducing agents.

MATERIALS AND METHODS

Animals.—The animals were outbred female Swiss mice, Syrian golden hamsters, and NMR rats from the Eppley colony. The mice and rats, 8 weeks old at the beginning of the experiment, were housed in plastic cages, 10 and 4/cage, respectively. Hamsters were 8–10 weeks old at the start and kept 4–6/cage. The animals were given pelleted diets (Wayne, Allied Mills, Chicago, Ill.) and water ad libitum.

Treatment.—The dorsal skin of the animals was shaved before irradiation from four fluorescent Westinghouse 40-W FS-40 T12 sunlamps (Westinghouse Large Lamp Division, Houston, Texas), emitting 1.8×10^7 ergs/cm^2/hour at a distance of 15 inches. Light energy was measured with an International Light IL 335 (International Light, Inc., Newburyport, Mass.) exposure meter. The animals were irradiated three times/week for 2 hours during a 4-week

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Abbreviations: W = watt(s); DMBA = 7,12-dimethylbenz[*a*]anthracene; TPA = phorbol myristate acetate; H & E = hematoxylin and eosin; TEM = transmission electron microscopy; SEM = scanning electron microscopy.

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[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] Supported by Public Health Service contracts 43-68-959 and N01-CP33278 from the Division of Cancer Cause and Prevention, National Cancer Institute.

[3] Eppley Institute for Research in Cancer, University of Nebraska Medical Center, 42d Street and Dewey Avenue, Omaha, Nebraska 68105. *Present address:* Department of Pathology, University of Kuopio, Harjulantie 1, 70100 Kuopio 10, Finland.

[4] I am grateful to J. Rowland and J. Franklin for their excellent technical assistance, to A. Washington and W. Williams for the photography, and J. Villareal for the illustrations.

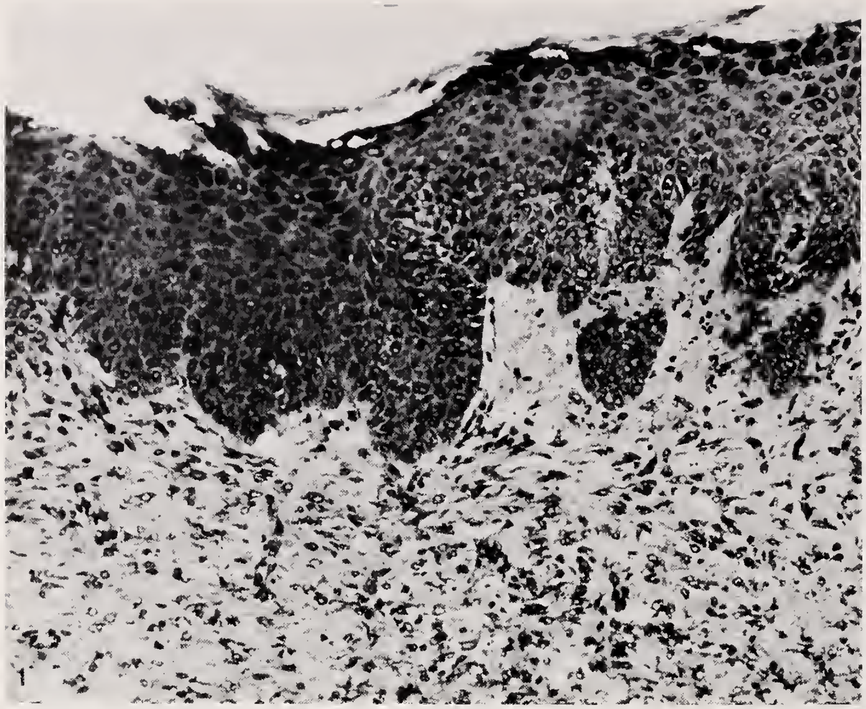


FIGURE 1.—Epithelial proliferation induced by UV irradiation consisting of an increased number of cells and cell layers in mouse skin. H & E. × 95

period. Also used was a high-voltage (6,000 W) xenon lamp (Atlas Electric Devices, Chicago, Ill.), emitting 1.7×10^7 ergs/cm^2/minute at a distance of 43 cm and a 100-W high-pressure mercury lamp (Zeiss, Wetzlar, Federal Republic of Germany). For tumor behavior studies, Swiss mice received repeated carcinogen treatment with 5 μg DMBA in acetone twice weekly for life on the shaved area of the skin with a precision pipette. Initiation was with either 30 μg once or 300 μg once of DMBA followed by promotion with 3.2 μg TPA in acetone twice weekly for 15 weeks. In single application studies, 300 μg DMBA was given once. All animals were checked weekly and tumors were recorded. The animals were killed at different intervals for tumor morphology studies. For tumor behavior studies, the animals were kept for life and allowed to die spontaneously.

Sections were examined from all skin lesions and organs showing gross abnormalities. Formalin-fixed sections were embedded in paraffin and stained with H & E, Van Gieson's, and other stains when necessary. For ultrastructural studies, skin specimens were cut into small blocks with a razor blade and fixed in glutaraldehyde, washed in cacodylate buffer, and postfixed with osmium tetroxide, followed by treatment with uranyl acetate. The specimens were embedded in an Araldite 502 epoxy resin mixture. Thick sections of 1 μ were cut from each block and the slides were stained with 0.2% toluidine blue and examined by light microscopy. TEM of thin sections, cut on a Porter-Blum MT-1 ultramicrotome (Sorrall Co., Newtown, Conn.) and contrasted with uranyl acetate and lead citrate, were examined at 60 kV in a Philips 300 electron microscope (Philips Electronics Instruments, Mahwah, N.J.).

Selected specimens were prepared by the method of Malick et al. (*9*) and studied by SEM. The specimens were fixed by the same procedures as for TEM in 2.5% glutaraldehyde and postfixed in osmium tetroxide. Subsequently, specimens were rinsed with six changes of distilled water during a 10- to 15-minute period. The next stage consisted of incubation in a 1% freshly prepared, filtered solution of thiocarbohydrazide in distilled water for 20–30 minutes at room temperature with gentle agitation. The tissues were then rinsed in six changes of distilled water and placed in 1% osmium tetroxide in distilled water for 2–3 hours at room temperature with gentle agitation. This process was repeated, and the tissues were subsequently dehydrated, dried by critical point, and mounted on SEM stubs. The specimens were analyzed in an ETEC Autoscan scanning electron microscope (ETEC Corp., Hayward, Calif.).

RESULTS

Morphologic Aspects

Tumors induced in the Swiss mice and Syrian golden hamsters followed the two main pathways of epidermal proliferation and neoplastic transformation or dermal tumor induction and growth. The first step in epidermal tumor formation consisted of squamous cell proliferation and increased numbers of squamous cells and cell layers, which gradually became papillomatous (fig. 1). This was accompanied by increased cellular atypia, nuclear enlargement, hyperchromatism, indentation, and prominent nucleoli (fig. 2). Maturation and keratinization were disturbed by intracellular keratin clumps, aggregations of tonofibrils, widened intercellular spaces and abnormal cellular attachments. Subsequent treatment caused disintegration of the basal membrane, which was seen even before formation of frank neoplasms (fig. 3). This basically proliferative response was frequently replaced by a dysplastic progression showing a solar, keratosis-like pattern with cellular pleomorphism, occasionally with pseudoepitheli-

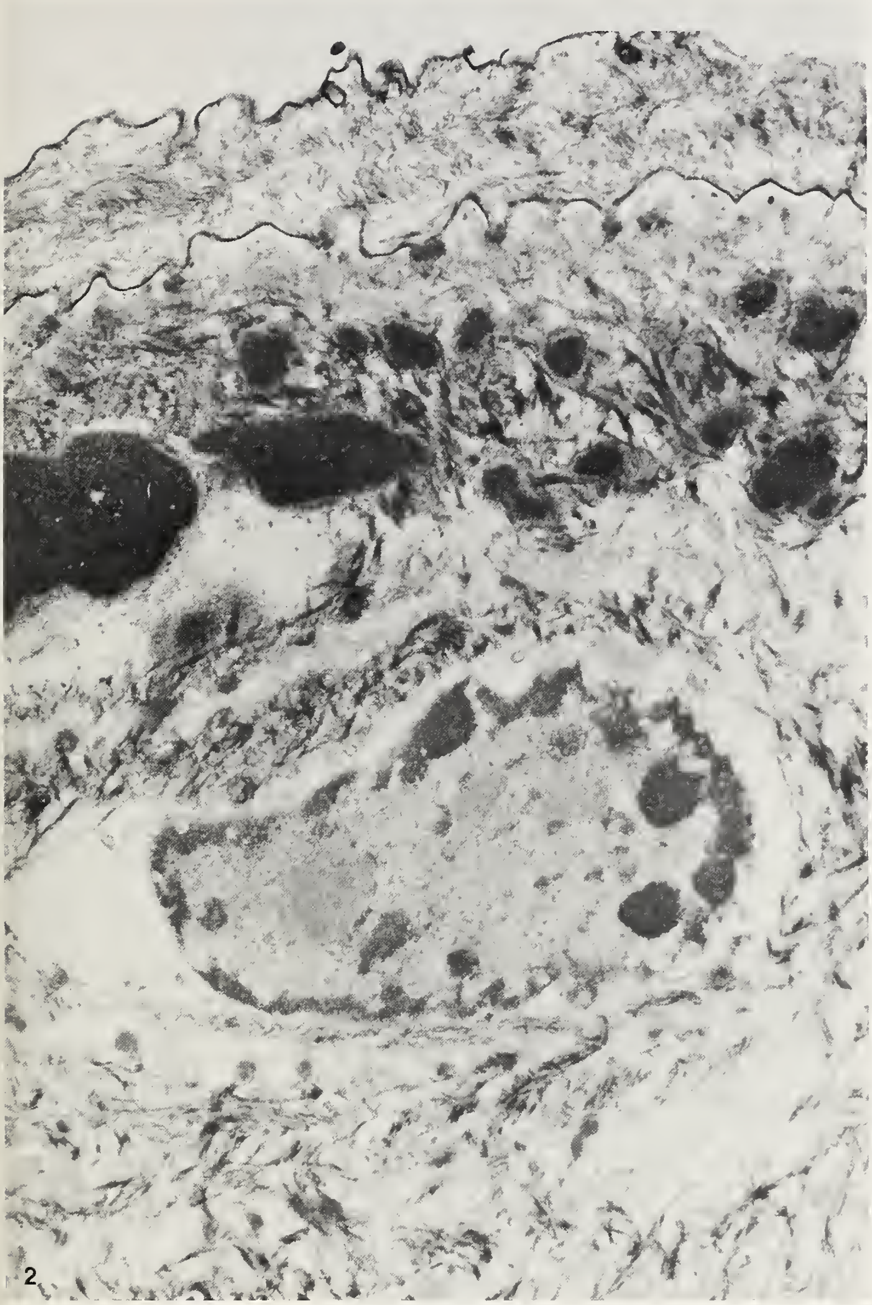


FIGURE 2.—Enlarged hyperchromatic nucleus, large keratin clumps and deranged epithelial maturation in UV-irradiated mouse skin. Uranyl acetate-lead citrate. × 6,000

omatous hyperplasia-like features and which ultimately invaded the dermis (fig. 4). The tumors first seen were papillomas (fig. 5), i.e., acanthopapillomas with a predominantly epithelial component and fibropapillomas, the latter consisting of a fibrous stroma covered by squamous epithelium. A varying degree of cytologic abnormalities accompanied tumor formation and nuclear and cytoplastic alterations, including the intramitochondrial dense body shown here (fig. 6).

The malignant neoplasms were ultimately squamous cell carcinomas of different types: solid, moderately differentiated, keratin horn-containing tumors, individually keratinizing tumors with distinct intercellular bridges, and less-differentiated, nonkeratinizing spindle cell tumors (fig. 7), in which ultrastructural analysis revealed squamous cell patterns (fig. 8).

Keratoacanthomas, proliferating epithelium on a cup-shaped base, are more frequent in UV-irradiated animals of different species: mice, rats, and hamsters, than in those treated with carcinogens (fig. 9).

Another type of neoplastic progression seen in mice, particularly after intensive treatment with large doses of UV light in a short time, consisted of ulceration, scarring, and subsequent formation of dermal tumors. These began as aggregates of regularly built, elongated cells with small, monomorphic nuclei (fig. 10). Epithelial proliferation was some-

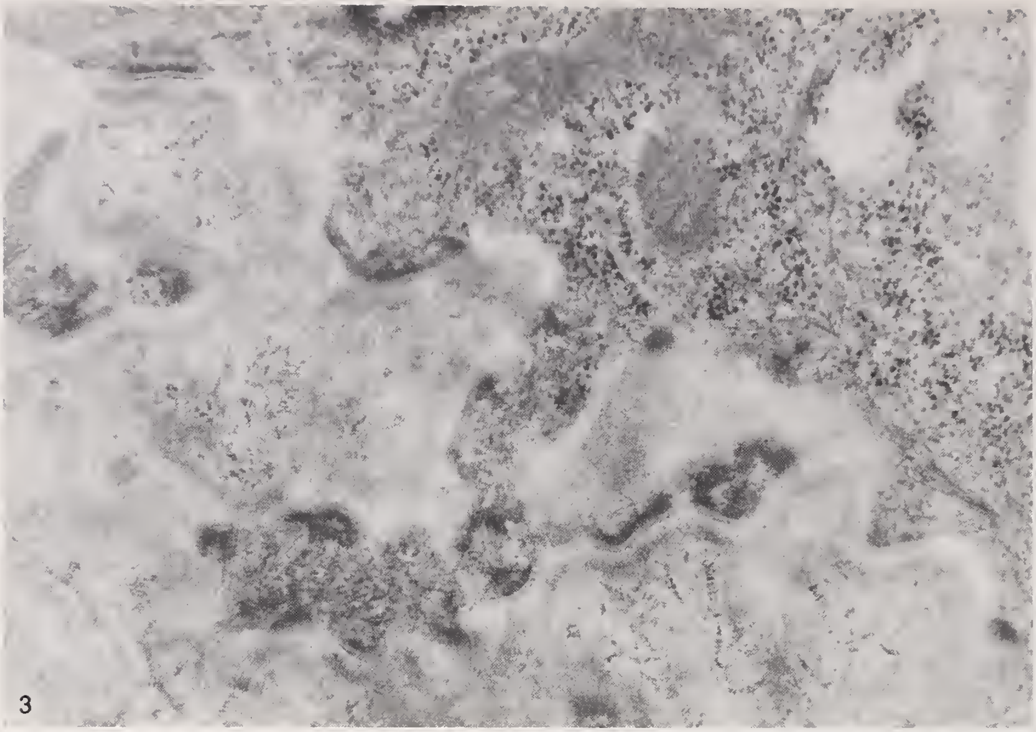


FIGURE 3.—Basal membrane disintegration in UV-irradiated mouse skin. Uranyl acetate-lead citrate. × 22,000

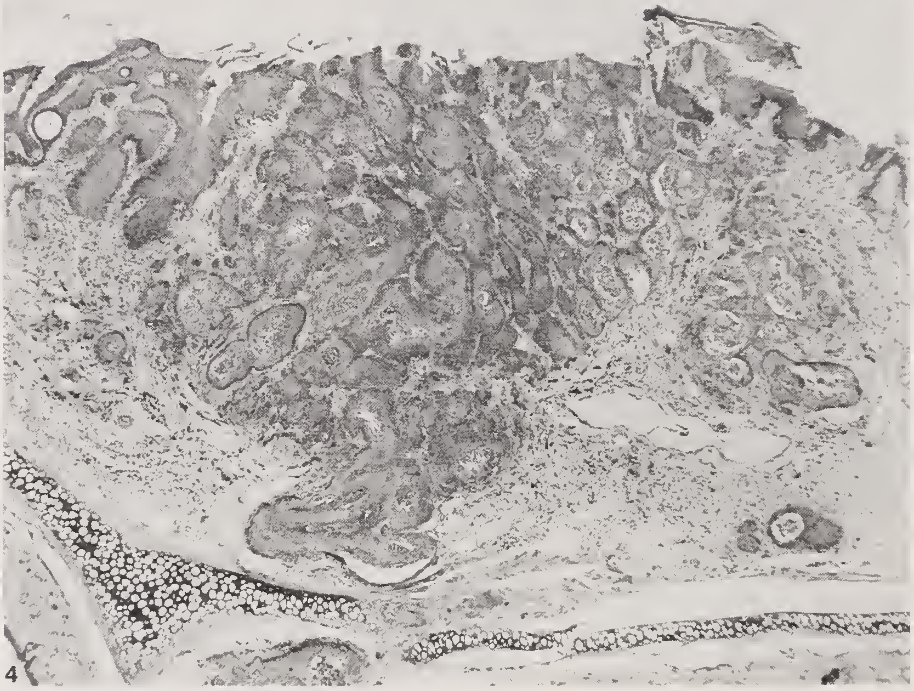


FIGURE 4.—Dysplastic epithelial alterations with large numbers of slightly atypical cells. H & E. × 95

times observed as a secondary phenomenon. Occasionally, the tumors extended grossly through the surface and had the appearance of a papilloma, although they consisted entirely of fibroblasts. Some were remarkably acellular and showed a prominent fibrillary pattern. The tumors were composed of polymorphic large cells with prominent nuclei. Ultrastructural analysis showed the predominant cellular components (fig. 11): a dark-cell type with hyperchromatic nuclei and scanty cytoplasm and a light-cell type with large nuclei and abundant cytoplasmic ribosomes. The same cell types were seen also in malignant tumors in which cellular polymorphism frequently was considerable, with nuclear atypism and enlargement, numerous nucleoli, and a generally disorganized arrange-

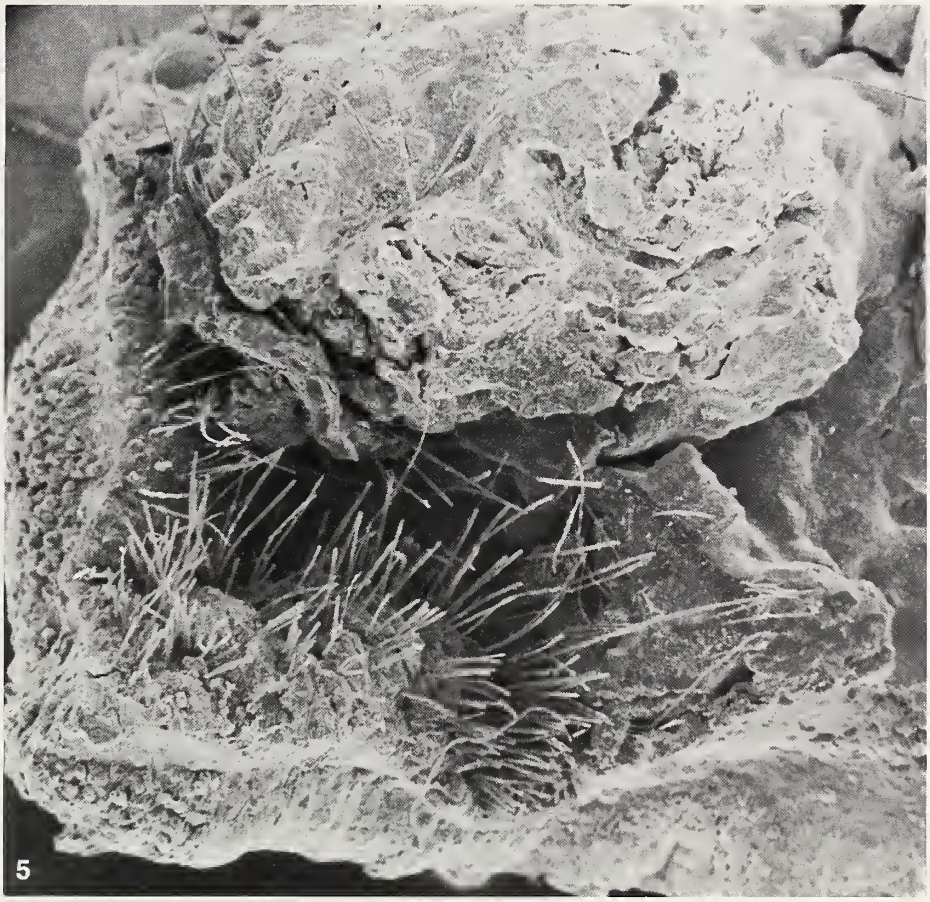


FIGURE 5.—Papilloma in UV-irradiated skin. Thiosemicarbazide. × 17

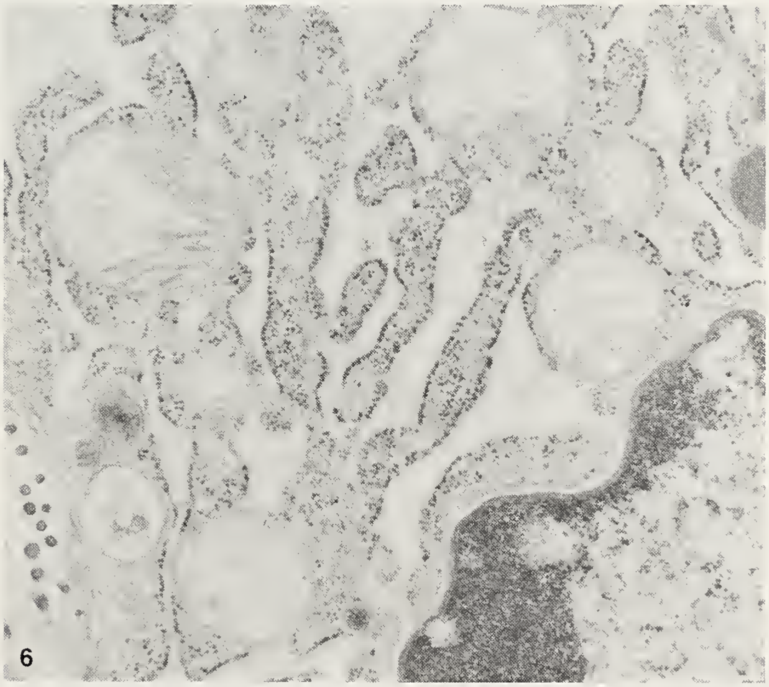


FIGURE 6.—Mitochondrial disorganization, splitting of cristae and intramitochondrial dense body in UV-irradiated mouse skin papilloma. Uranyl acetate-lead citrate. × 28,000

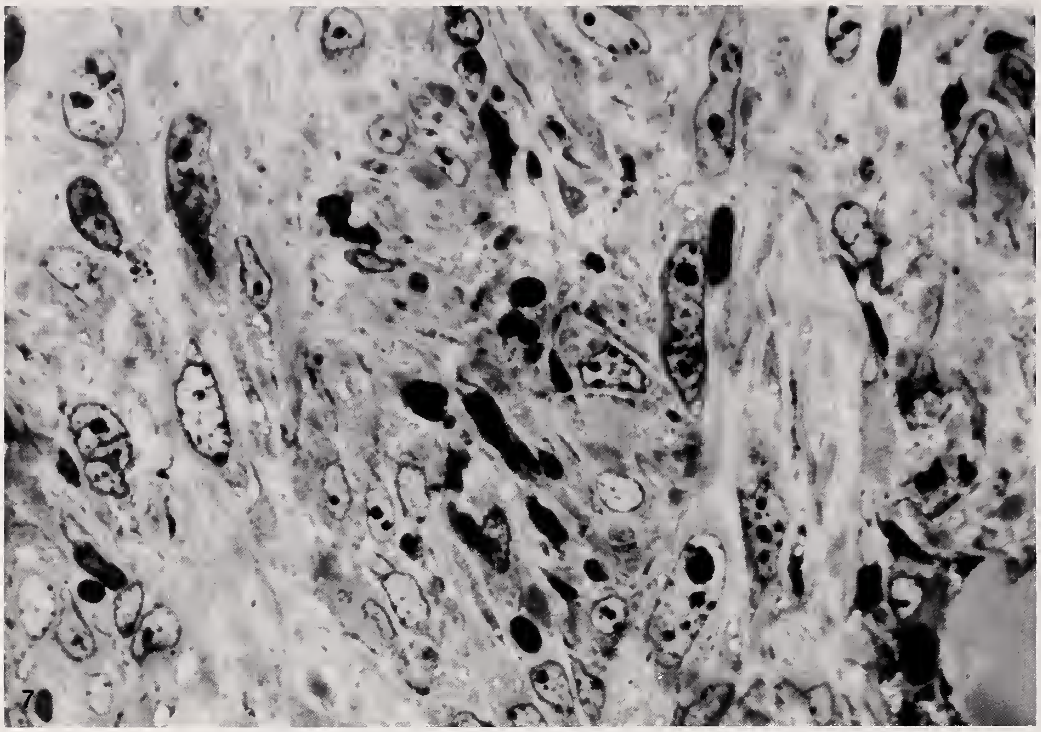


FIGURE 7.—Undifferentiated squamous cell carcinoma consisting of polymorphic cells in irregular arrangement. Toluidine blue. × 720

ment (fig. 12). The average life-span for animals with sarcomas was 49 weeks, compared with 45 weeks for animals with epithelial tumors.

Carcinosarcomas with neoplastic squamous and fibrous components were rare. However, many tumors initially classified in this group, when examined under TEM, proved to be anaplastic squamous cell carcinomas, with a prominent desmoplastic stromal reaction. Occasionally, we observed "collision" tumors consisting of a squamous cell carcinoma and a fibrosarcoma side by side (fig. 13).

A type of infrequently found, dermal neoplastic alteration in several animal species, i.e., mice, rats, and hamsters, is the vascular tumor, which usually begins as a proliferation of dilated vascular spaces (fig. 14) with regular endothelial lining (fig. 15). The endothelium infrequently proliferates to form angiosarcomas or invasive tumors composed of large atypical cells arranged in a nodular pattern (fig. 16).

As shown previously, adnexal tumor formation was not as widespread in UV-treated animals as in carcinogen-treated rats. Hyperplasia and cystic disorganization of hair follicle walls (fig. 17) was common but rarely progressed to grossly visible neoplasia. Trichoepitheliomas with barely visible follicular arrangements were rarely seen (fig. 18). Even more uncommon were hamartoid tumors: hair follicle-derived trichofolliculomas or sebaceous gland tumors. Sebaceous gland epitheliomas and carcinomas were even more rare.

Research on the induction of pigmented tumors has been less successful. Benign dermal melanocytic lesions or blue nevi, observed in hairless mice, were similar to those previously reported (*10*). They were grossly seen as papules, 2–20 mm in diameter, and histologically composed of tightly arranged, intensive, pigment-laden polyhedral cells. Subcutaneous arrangements of pigmented cells also were in pigmented animal strains and in the skin of the Syrian golden hamster. In our studies, these tumors were apparently spontaneous because the frequency was low (about 4%) and unrelated to treatment. These neoplasms, composed of polyhedral or spindle-form cells arranged in a whorl-like pattern, began as hyperplasia of perifollicular melanocytes and developed by spreading both laterally and deeply into the dermis. These tumors did not show junctional activity or metastases and rarely killed the host.

The histologic tumor types are summarized in table 1. Epidermal benign tumors were most frequently seen in the dorsal skin of 45% of the Syrian golden hamsters and in the ears of 30% of the rats. Squamous cell carcinomas usually developed in the mouse and rat ear (in 5–10% of the animals); undifferentiated types were more common. Appendage tumors were infrequently observed among the different species, but the incidence never exceeded 5%. Benign and malignant dermal tumors were most frequently noted in the mouse dorsal skin (20%) and the ear (25% of the animals). Vascular tumors occurred in the ears of the mice and rats in incidences varying from 5–10%. We observed pigmented tumors, blue nevi, in 10–20% of the hairless mice and melanocytomas in less than 2% of the hamsters.

Biologic Aspects

Evaluation of tumor incidence in relation to the inducing agent showed UV irradiation was significantly weaker in tumor numbers (text-fig. 1) and number of tumors per tumor-bearing animal than after repeated carcinogen or initiation–promotion treatment, although the total of tumor-bearing animals was fairly high (text-fig. 2). These figures also indicate the low actual number of tumors and the number of tumors grossly observed at any given moment.

These data emphasize the need for an estimation of the

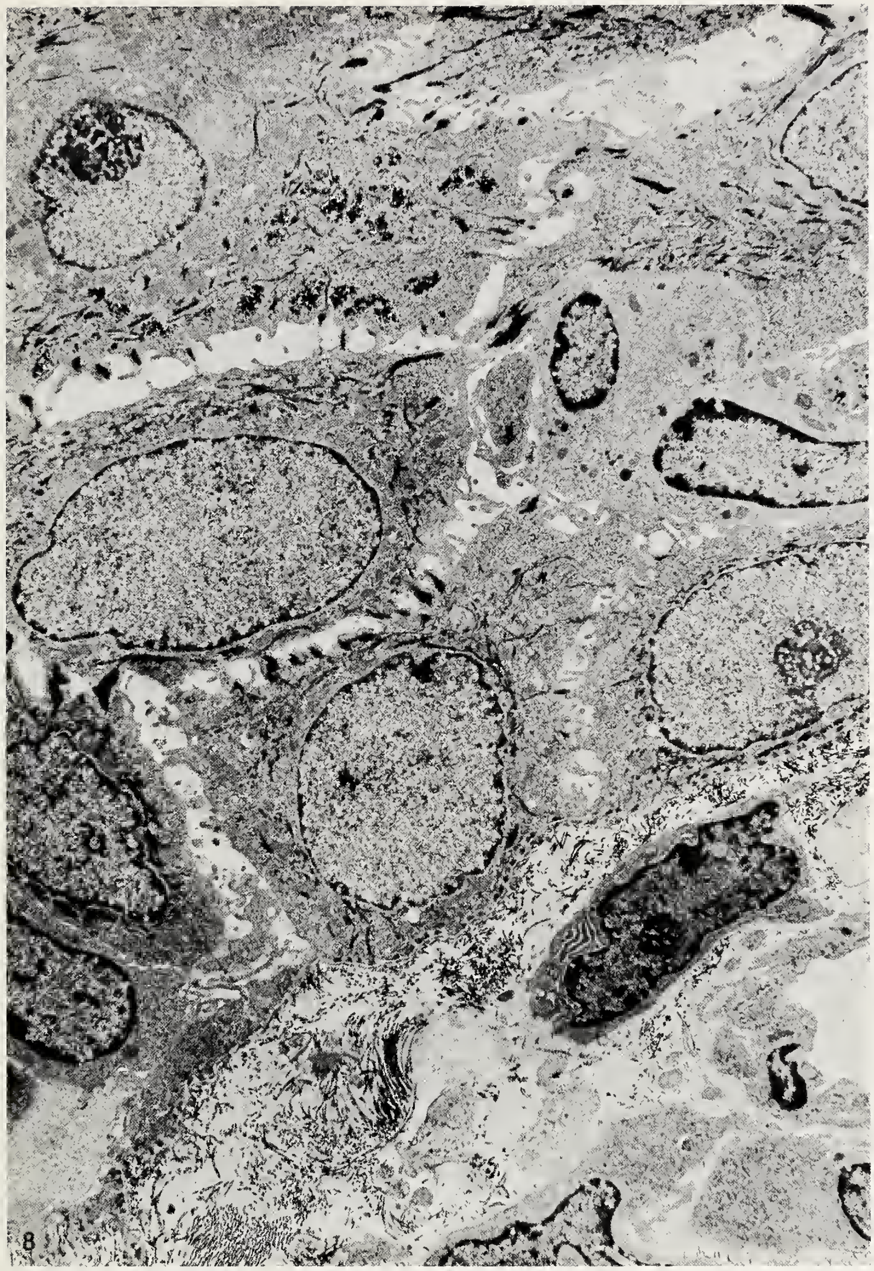


FIGURE 8.—High-power view of squamous cell carcinoma showing widened intercellular spaces, deranged intercellular attachments and cytoplasmic organelles. Uranyl acetate-lead-citrate. × 6,000

animals-at-risk compared with the number of animals alive and therefore still prone to tumor development. Few studies published thus far on UV carcinogenesis use the life table method, which accounts for survival in estimations on tumorigenesis. As shown here (text-fig. 2), UV irradiation significantly lowered the survival rate, thereby decreasing the animals-at-risk. The actual number of tumor-bearing animals (animals alive with tumors) at any given moment was fairly low, as most tumor-bearing animals died a short time after the tumor appeared.

The same observations can be made in animals that received a single application of polycyclic hydrocarbon (text-fig. 3), which can cause a decreased survival rate and may account for the small number of tumors appearing late in the experiment. This also shows the persistence of neoplastic transformation and the long latency period for tumor manifestation.

Skin tumor induction by the two-stage method repeatedly caused formation of tumors in animals either in a single one-time dose of 300 μg DMBA (text-fig. 3), followed by a repeated TPA treatment, or in 1 noncarcinogenic dose of 30 μg DMBA alone (text-fig. 4) The smaller dose was actually more effective and resulted in a greater number of tumor-bearing animals due to the higher survival rate. In this instance, also, tumors formed after cessation of treatment. Early in the experiment, most tumor-bearing animals were alive, whereas later the less-susceptible animals were alive.

Repeated carcinogen treatment was the most effective tumor inducer and caused tumors in nearly all animals (text-fig. 5). Also, the number of animals alive with tumors at any

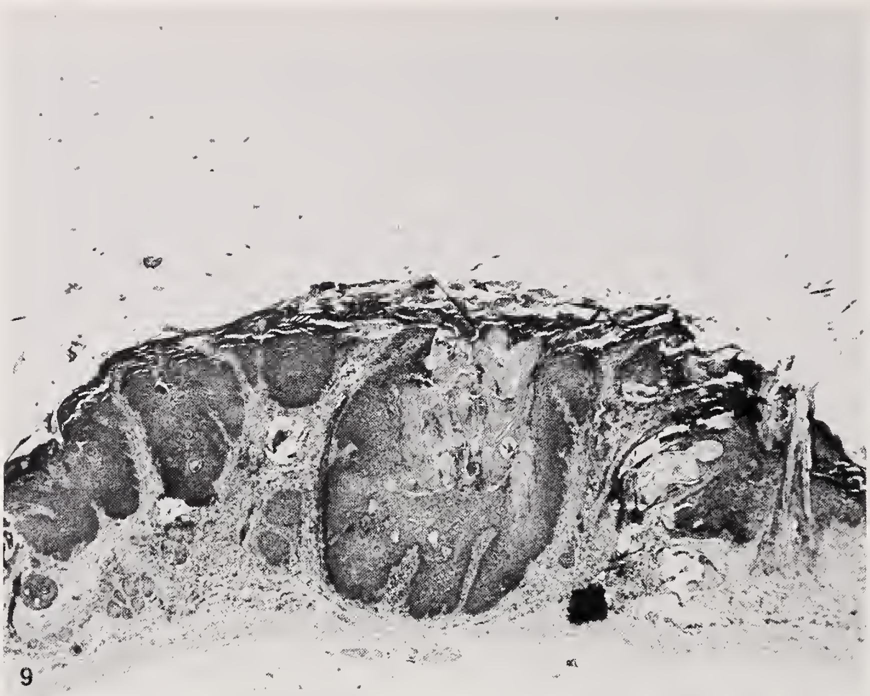


FIGURE 9.—Keratoacanthoma in UV-irradiated Syrian golden hamster. H & E. × 30

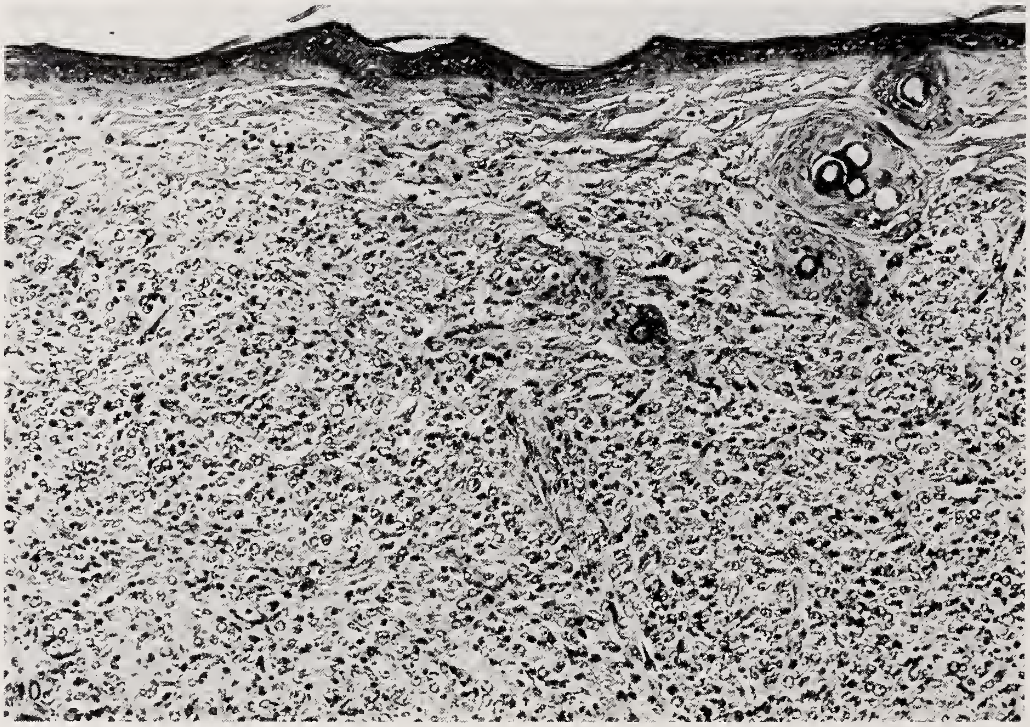


FIGURE 10.—UV-induced dermal tumor composed of small regular cells. H & E. × 150

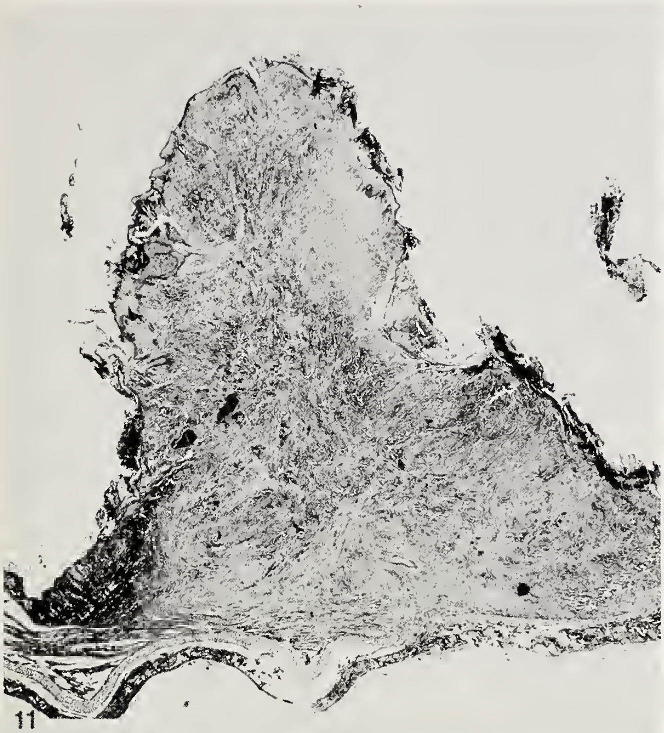


FIGURE 11.—Fibroma growing exophytically to the surface. H & E. × 9.5

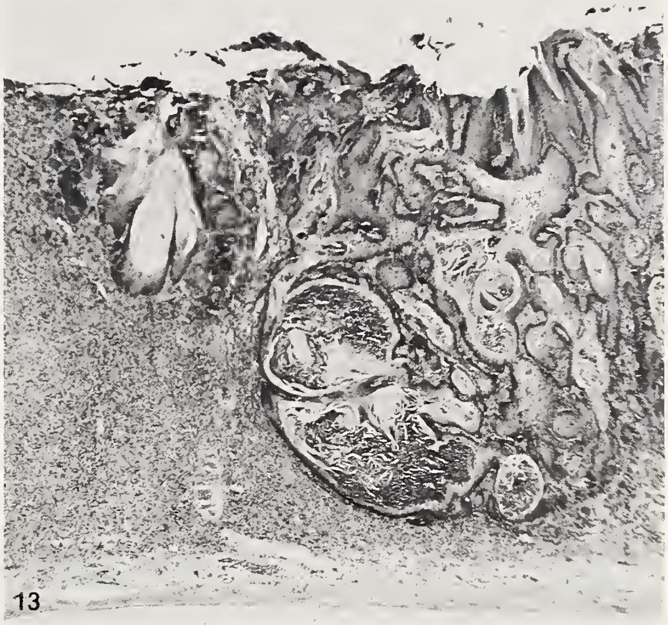


FIGURE 13.—Collision tumor composed of squamous cell carcinoma and fibrosarcoma in UV-irradiated mouse skin. H & E. × 60

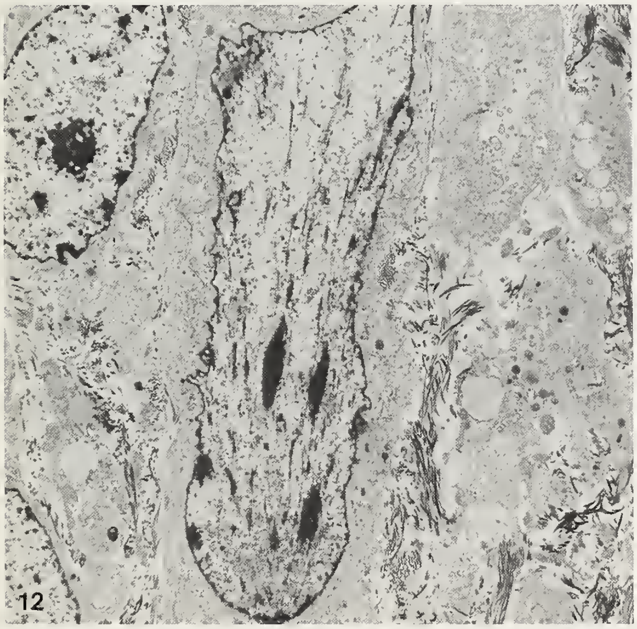


FIGURE 12.—High-power view of fibrosarcoma showing light and dark cells. Uranyl acetate-lead. × 28,000

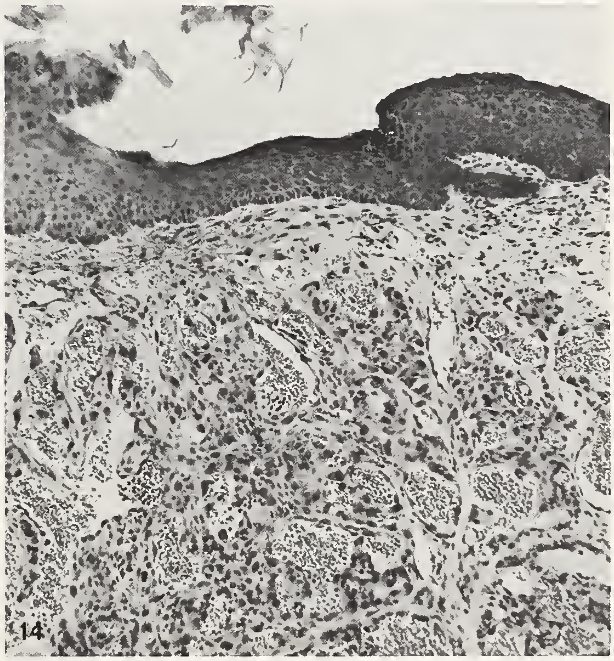


FIGURE 14.—Hemangioma composed of blood-filled vascular spaces in UV-irradiated mouse skin. H & E. × 60

given period was small. This figure also showed a dose–response relationship, with the percentage of tumor-free animals decreasing from 76% at 35 weeks to 25% at 40 weeks and to 2% at 55 weeks. These results emphasize the difference between two-stage carcinogenesis and repeated carcinogen application in skin tumor formation and the relationship between UV irradiation and carcinogen-induced neoplastic transformation and manifestation.

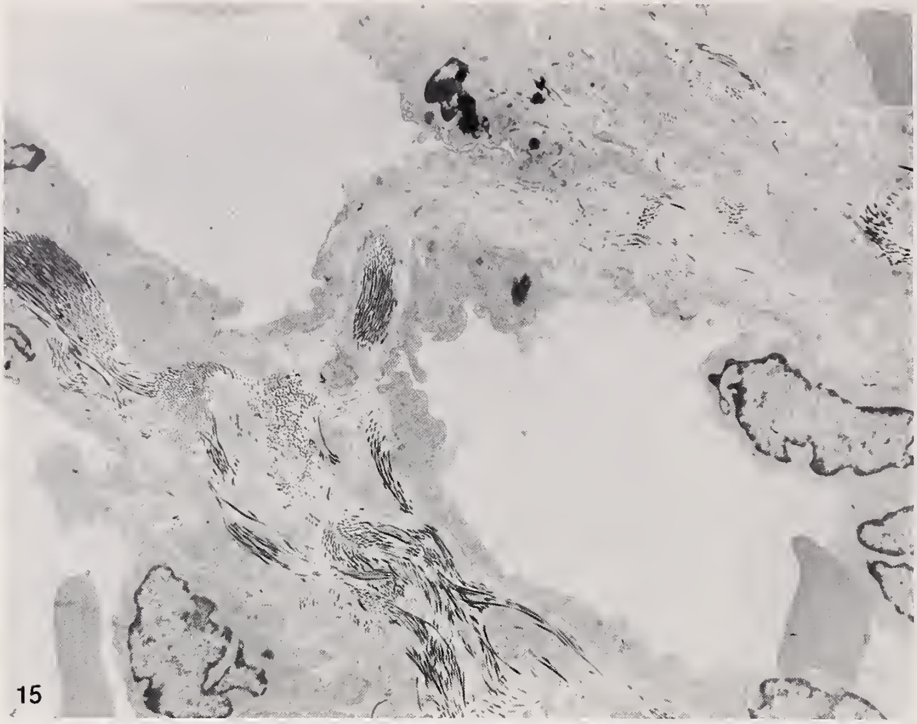


FIGURE 15.—High-power view of hemangioma showing regular endothelial cells surrounding vascular spaces. Uranyl acetate-lead-citrate. × 12,000

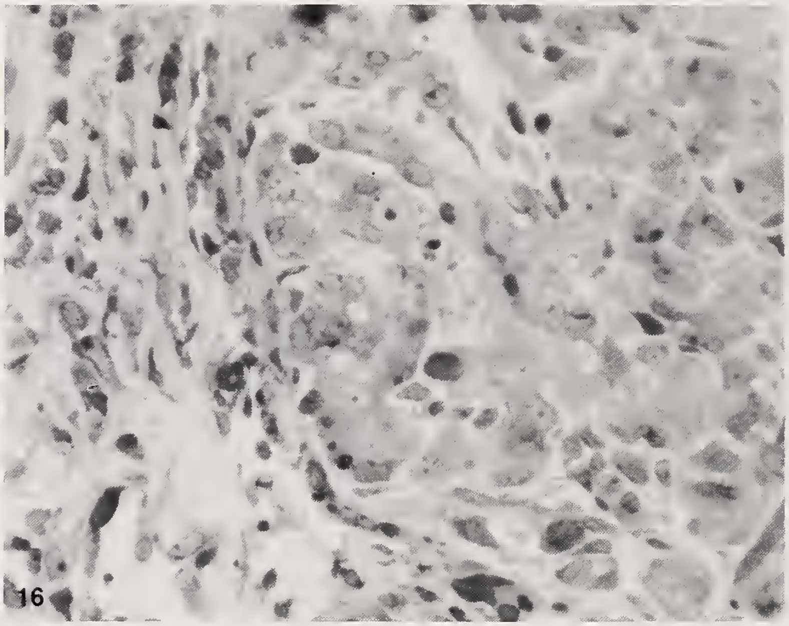


FIGURE 16.—Angiosarcoma composed of polymorphic cells arranged in nodular pattern induced by UV-irradiation of mouse skin. Toluidine blue. × 450

DISCUSSION

As shown in these studies, the response of carcinogen-treated animals to neoplastic and toxic stimuli may vary in relation to their immunologic and general conditions. The animals could be categorized as follows: 1) alive without tumors but at risk for subsequent tumor formation, 2) having tumors but liable to formation of additional tumors, 3) dying without tumors, and 4) dying with tumors. A high dose level increased the tumor incidence but caused a decreased number of animals-at-risk, whereas prolonged exposure resulted in an increased influence of unrelated factors, i.e., infection. Repeated carcinogen treatment in mice was effective and induced a high percentage of tumor-bearing animals, although at any given time, the actual number of tumors was small. The probable reason for this was the death of animals from the toxicity of the treatment. UV irradiation was less effective in inducing tumors but also caused a less severe reduction in the survival rate. UV light, as the initiating agent, was particularly weak and induced a minimal number of tumors (*4, 11*).

Studies on tumor morphology induced by artificial light

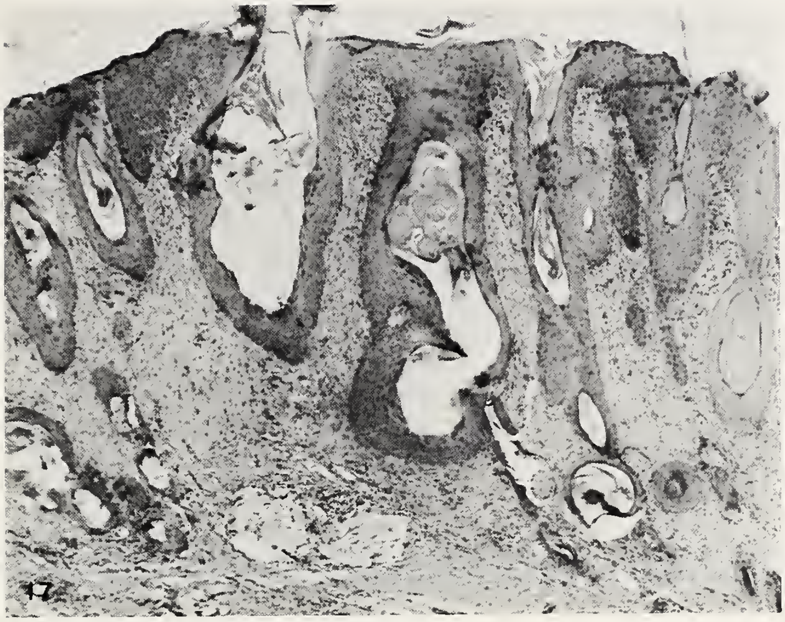


FIGURE 17.—Hyperplastic follicular walls of UV-irradiated mouse epidermis. H & E. × 37

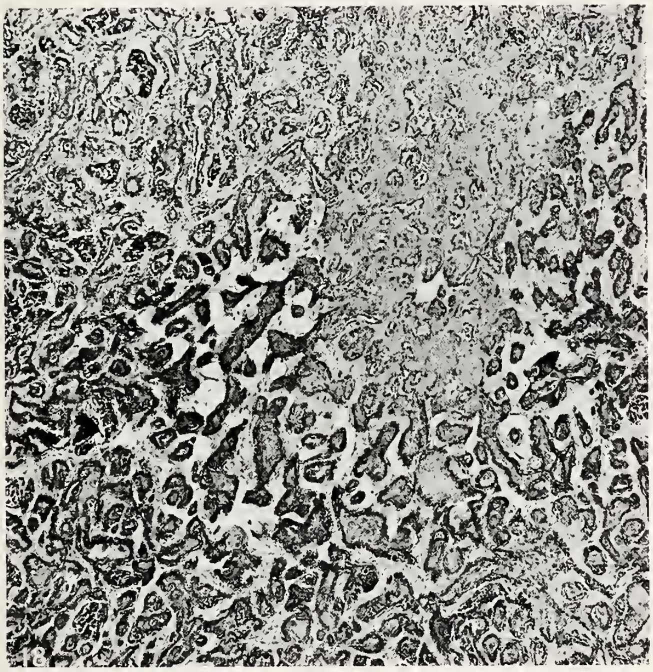


FIGURE 18.—Follicular tumor in mouse skin composed of elongated cells in a whorl-like pattern. H & E. × 48

sources in animals primarily described the occurrence of subcutaneous fibromas and fibrosarcomas (*1, 12, 13*). Early investigators (*14*) reported undifferentiated spindle cell tumors that were regarded as carcinosarcomas and sarcomas in rats. Grady et al. (*12*) found that irradiation of mice induced mainly sarcomas; of 112 altogether, only 3 were carcinomas and 25 were carcinosarcomas. Sarcoma induction is partly species-specific, since these tumors are not seen in UV-irradiated hairless mice (*1*), Syrian hamsters (*5, 15*), or guinea pigs (*5, 15*).

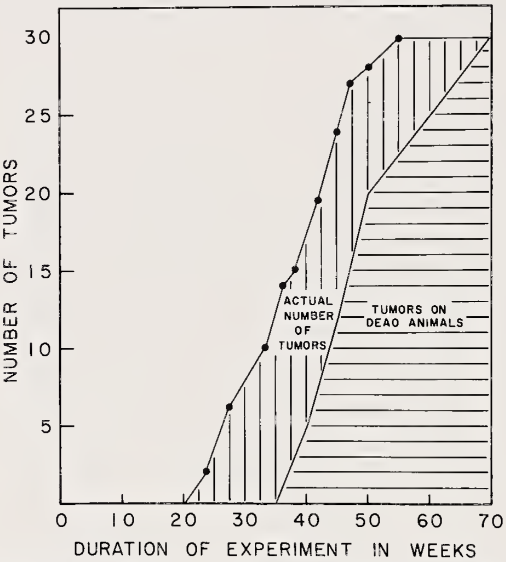
The differences in distribution of tumor types, with sarcomatous growth predominating in mice and carcinomas in man (*16, 17*), may be explained partly by variations in UV light penetration, with a greater amount reaching the mouse dermis (*8, 18*). Mice have a morphologic epidermal structure different from that of man, and it may be necessary to assume that the connective tissue of mouse dermis is more susceptible to tumor induction by UV light or, alternatively, that sarcomas develop earlier than carcinomas, as seen here. The ulcerogenic effect of large doses of UV irradiation in adnexal skin has also been associated with fibroma and sarcoma formation, the surface ulcer allowing not only for greater penetration but also for the irritant effect observed under these experimental conditions (*5, 15*).

The relationship between tumor-inducing treatments, summarized in text-figure 6, is based on these results and those previously published (*5, 6, 15*).

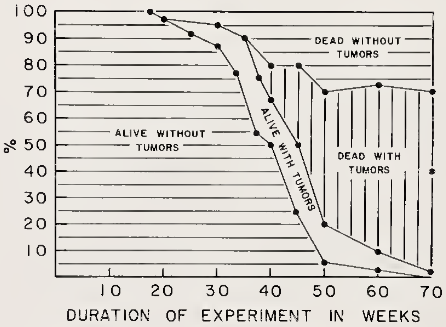
Epidermal tumors are easily induced by a variety of agents in mouse skin (*6*). Winkelman et al. (*2*) reported production of squamous cell carcinomas on the backs of hairless mice with UV light. Further studies established that carcinomas can be induced in this animal, almost to the exclusion of sarcoma formation (*1*). Winkelman et al. (*3*) followed the tumor progression histologically from premalignant changes through the frank, invasive cancer stage. In the early thirties, investigators reported epithelial tumors in rats and mice (*14, 19, 20*), although later, the dermal tumor response to UV irradiation predominated. No mention was made of skin sarcomas by Beard et al. (*21*) in their studies with albino rats. Their findings in 12 animals included: 9 carcinomas of the external ear, 6 sarcomas of the eye, and 1 carcinoma of the nose skin.

TABLE 1.—*Histologic types of tumors induced by UV light*

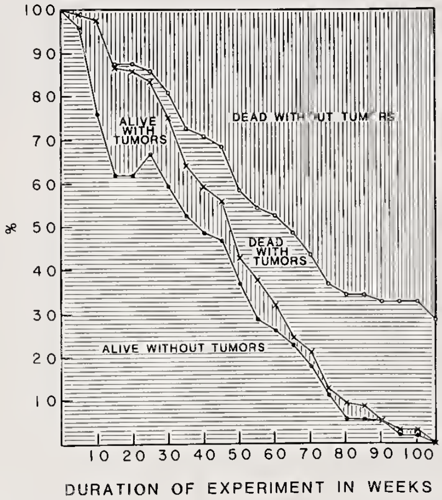
Epidermal	Appendage	Dermal	Pigmented
Papilloma	Sebaceous epithelioma	Fibroma	Blue nevus
Acanthopapilloma	Sebaceous carcinoma	Fibrosarcoma	Nevocytoma
Fibropapilloma	Trichofolliculoma	Angioma	
Pilar cyst	Trichepithelioma	Angiosarcoma	
Epidermal cyst	Basal cell carcinoma		
Keratoacanthoma	Solid		
Solar keratosis	Pilar		
Atrophic	Keratotic		
Hypertrophic			
Pseudocarcinomatous hyperplasia			
Squamous cell carcinoma			
Keratotic			
Solid			
Nonkeratinizing			
Spindle cell			
Pseudoglandular			
Carcinosarcoma			



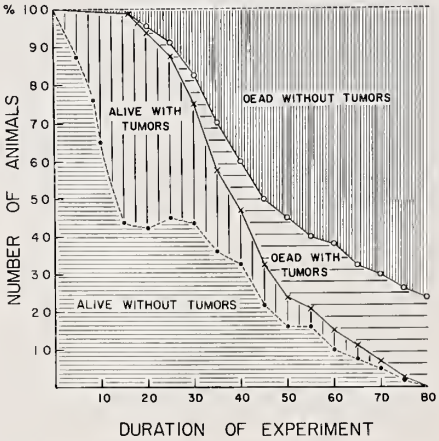
TEXT-FIGURE 1.—Effect of UV irradiation on mouse skin: total tumors, clinical number, and tumors in dead animals.



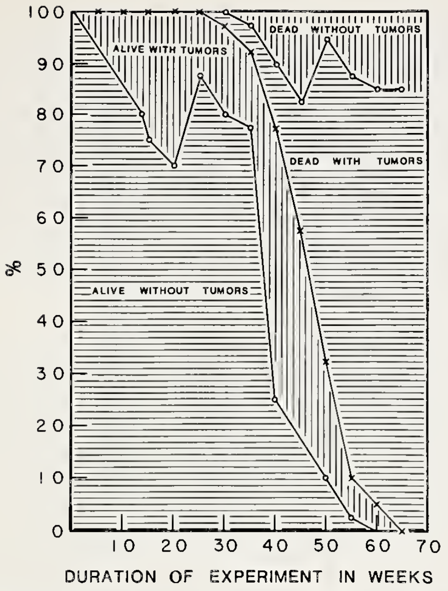
TEXT-FIGURE 2.—Distribution of animals in group given UV light repeatedly.



TEXT-FIGURE 3.—Distribution of animals given 300 μg DMBA once. x——x = survival rate; ●- - -● = tumor-free animals; ○——○ dead tumor-bearing animals.



TEXT-FIGURE 4.—Distribution of animals in group given 300 μg DMBA followed by TPA repeatedly. x——x = survival rate; = ●- - -● tumor-free animals; ○——○ = dead animals bearing tumors. Duration of experiment is given in wk.



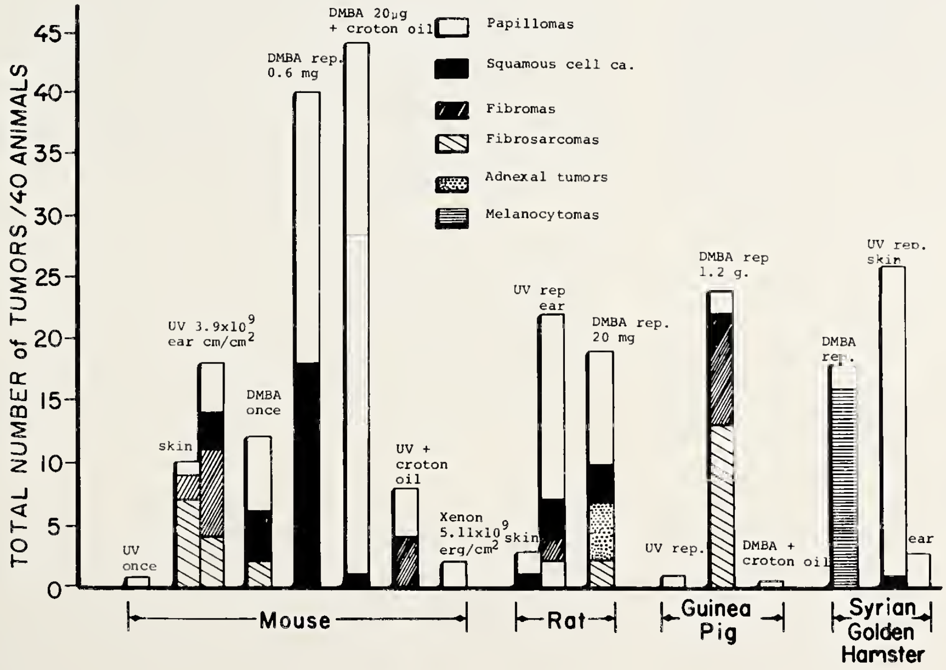
TEXT-FIGURE 5.—Distribution of animals in a group given 30 μg DMBA and TPA repeatedly. x——x = survival rate; ○——○ = tumor-free animals.

The influence of the stroma on epidermal tumor development was emphasized by several investigators (*22, 23*). The striking, sun-induced dermal change, described as actinic elastosis, has frequently been correlated with skin cancer formation. However, Sams et al. (*24*) demonstrated that actinic elastosis is not essential for development of basal cell epithelioma in man. In addition, experimental production of elastosis in animals exposed to UV light was not associated with cancer formation (*24*). Conversely, experimental UV light-induced skin cancers have not shown these striking dermal elastic tissue reactions (*1, 3*). Thus actinic elastosis is probably a concomitant finding rather than a specific influence in sun-induced skin cancer.

Adnexal tumors common in carcinogen-treated rats (*6, 25*) are infrequent in UV-irradiated animals (*5, 15*), despite the obvious follicular alterations seen also in this study. Previously, only a small number of basal cell carcinomas were reported (*14*).

Pigmented tumors, the most interesting type, epidemiologically, are seen infrequently in animals. Melanocytomas are easily induced by chemical carcinogen treatment, as shown by Shubik et al. (*26, 27*). Epstein and Epstein (*1*) reported a high incidence of blue papules composed of pigmented cells (blue nevi) in hairless mice but no melanomas. The same phenomenon was also observed in our work. Fortner and Allen (*28*) reported the occurrence of spontaneous melanomas, similar to those in man, in hamsters. Their sensitivity to UV irradiation is unknown (*28*) and the effect of pigment, in general, received scanty interest. Freeman (*29*) used a pigmented rat strain and induced tumors in 67% of the animals, with an average latency period of 193 days, whereas albinos had an 8% tumor incidence and a 283-day latency period.

The tumors, summarized in table 1, show the different types of neoplastic response encountered in animal skin subsequent to UV irradiation. As shown before, however, the process depends greatly on the experimental conditions. Care must be exercised in designing proper studies in UV carcinogenesis, in analyzing results, and particularly in making comparisons with the human situation. More research is needed



TEXT-FIGURE 6.—Effect of various types of carcinogen treatment on animals of different species and strains.

to evaluate the situation fully, especially with regard to malignant melanomas, morphology, biology, and experimental induction.

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Ultraviolet Light in the Oncogenic Transformation of Cultured C3H/10T1/2 Mouse Embryo Cells[1]

Sukdeb Mondal *and* Charles Heidelberger[2]

ABSTRACT—Skin tumors are produced in rats, mice, and humans by exposure to UV light. We developed a mouse embryo fibroblast line C3H/10T1/2, which can be transformed by chemical carcinogens, X-irradiation, UV radiation, and oncornavirus. When we irradiated these cells with 10, 25, 50, 100, 150, or 200 ergs/mm^2 of UV light, neither transformation nor cytotoxicity was observed at the two lower doses. When the irradiated cells were cultured in medium containing 0.1 μg TPA/1 ml (a potent tumor promoter) starting from 0–120 hours after irradiation, a high frequency of transformation was always produced. When the cells were initiated with subeffective concentrations of 0.1 μg MCA/ml followed by UV radiation at different intervals, no transformation occurred; however, these initiated cells were transformed after TPA treatment. When we treated the cells with multiple exposures to UV light, no transformation occurred then nor when the cells were treated with TPA followed by UV irradiation at different intervals. Thus UV in our system acts as a pure initiator in the two-stage process of oncogenic transformation.—Natl Cancer Inst Monogr 50: 71–73, 1978.

Skin tumors are produced in rats and mice by repeated exposures to UV light (*1–4*). Following a single exposure of mouse skin to UV light, tumor development was promoted by repeated application of Croton oil (*5*). This is a typical example of two-stage carcinogenesis as originally described by Berenblum (*6*), Berenblum and Haran-Ghera (*7*), and Mottram (*8*). In our laboratory, we developed C3H/10T1/2 Cl 8, a cloned line of C3H mouse fibroblasts, which is nonmalignant and highly sensitive to postconfluence inhibition of cell division (*9*). This cell line undergoes oncogenic transformation by various carcinogenic chemicals (*10*), X-irradiation (*11*), and UV light (*12, 13*). With this cell line, we also demonstrated two-stage oncogenesis in culture (*14*), using several carcinogenic polycyclic hydrocarbons as initiating agents and a noncytotoxic concentration of TPA, an active promoter isolated from Croton oil (*15*). We could not transform our cells with either a single exposure or repeated exposures to UV light. We used the two-stage method to transform our cells, with various single doses of UV light as the initiator and TPA as the promoter (*13*).

MATERIALS AND METHODS

Early passages (4–10) of C3H/10T1/2 cells were plated in 60-mm Falcon dishes (2,000 cells) in 5 ml of Eagle's basal minimum essential medium and 10% heat-inactivated fetal calf serum (Grand Island Biological Co., Grand Island, N.Y.). Twenty-four hours later, the medium was removed, and the cells were irradiated once with a UV lamp (General Electric Co., Wilmington, Mass.), with doses from 10 to 200 ergs/mm^2. The dose of UVR was measured by an ergometer (Ultra-Violet Products, Inc., San Gabriel, Calif.) in ergs/square millimeter/second. After irradiation, the cells were fed with normal medium and were placed in a humidified incubator (Forma Scientific, Inc., Marietta, Ohio) at 37° C in an atmosphere of 5% CO_2 in air. At various times after irradiation (starting from 0 to 120 hr), some of the cultures were given 0.1 μg TPA/ml in the medium for the remainder of the experiment, i.e., up to 6 weeks. TPA (Consolidated Midland, Brewster, N.Y.), dissolved in spectrograde acetone and stored in amber bottles at −20° C, was added to the medium so that the final concentration of acetone was 0.5%. The control cultures received only 0.5% acetone in the medium. The cytotoxicity of the UV irradiation was determined by its effect on plating efficiencies of 200 cells, and the colonies were counted after 8–10 days. The dishes for transformation experiments were fixed in methanol and stained with 10% Giemsa after 5 weeks of continuous culture. Only type III foci (*10*) were counted to determine the transformation frequencies.

RESULTS

The results are shown in tables 1 and 2. The plating efficiencies indicate that UV irradiation at 10 and 25 ergs/mm^2 was not cytotoxic; however, larger doses were. No type III transformed foci were obtained in the acetone and TPA-treated control dishes. None of the UV-treated dishes showed any type III transformed foci at any dose. Many type I and type II foci were in several dishes treated with 50 ergs/mm^2 and more that we did not score as transformed, since the cells from these foci seldom produced tumors when injected into syngeneic hosts (*10*). When TPA was added to the medium of UV-irradiated cells, significant numbers of type III transformed foci developed at each dose, irrespective of the time of addition of TPA. It is evident, except with 10 ergs/mm^2, that addition of TPA between 48 and 120 hours after irradiation produced the highest transformation frequency. Also apparent is the fact that the number of dishes with type III foci was maximal at 25- and 50-erg/mm^2 doses and decreased

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Abbreviations: UVR = ultraviolet radiation; TPA = tetradecanoyl phorbol acetate; MCA = 3-methylcholanthrene.

[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] University of Southern California Cancer Center, Los Angeles, California 90031.

TABLE 1.—*Toxicity and percent of dishes of C3H/10T1/2 cells with transformed type III foci at doses of UV light and UV light + TPA*

Treatment schedule	Time, hr	Plating efficiency, %	No. of dishes with type III foci/total No. of dishes	Percent of dishes with type III foci
Acetone - 0.5%		19	0/35	0
TPA (0.1 μg/ml)		20	0/36	0
10 ergs/mm^2		19	0/24	0
+ TPA	0	19	3/10	30
+ TPA	24	19	2/11	18
+ TPA	48	19	2/12	16.6
+ TPA	96	19	4/12	33
+ TPA	120	19	3/12	25
25 ergs/mm^2		18	−0/39	0
+ TPA	0	18	4/24	17
+ TP	24	18	7/23	30
+ TPA	48	18	13/24	54
+ TPA	76	18	15/23	65
+ TP	96	18	14/22	64
+ TPA	120	18	15/24	63
50 ergs/mm^2		8	0/38	0
+ TPA	0	8	1/11	9
+ TPA	24	18	2/12	17
+ TPA	48	8	8/12	67
+ TPA	72	8	8/12	67
+ TPA	96	8	7/12	58
+ TPA	120	8	5/11	45
100 ergs/mm^2		1	0/35	0
+ TPA	0	1	1/10	10
+ TPA	24	1	7/22	32
+ TPA	48	1	6/15	40
+ TPA	72	1	8/19	42
+ TPA	96	1	3/12	25
+ TPA	120	1	3/11	27
150 ergs/mm^2		0.6	0/20	0
+ TPA	0	0.6	2/10	20
+ TPA	24	0.6	2/10	20
200 ergs/mm^2		0.3	0/25	0
+ TPA	0	0.3	0/12	0
+ TPA	24	0.3	1/11	9

TABLE 2.—*Experiments showing UV light as a pure initiator*

Treatment schedule[a]	No. of dishes with type III foci/total No. of dishes	Percent of dishes with type III foci
Acetone control, daily medium change until confluent	0/12	0
UV, 25 ergs/mm^2	0/12	0
UV, 25 ergs/mm^2 24 hr later	0/11	0
UV, 25 ergs/mm^2 daily until confluent, then once weekly (total 16 doses)	0/12	0
Two UV treatments of 50 ergs/mm^2 at weekly intervals then 2 × 100 ergs/mm^2 at weekly intervals, then 2 × 200 ergs/mm^2 at weekly intervals (total 6 doses)	0/12	0
UV, 25 ergs/mm^2 + TPA (120 hr)	6/12	50
UV, 25 ergs/mm^2 + MCA 48 hr later	0/12	0
MCA	0/10	0
MCA + TPA 48 hr later	6/10	60
TPA for 24 hr, then UV 25 ergs/mm^2	0/12	0
TPA for 120 hr then UV 25 ergs/mm^2	0/12	0

[a] The amount of MCA added was 0.1 μg/ml.

above 100 ergs/mm^2. Double or multiple doses of 25 ergs/mm^2 did not transform the cells, nor was transformation obtained when the dose of UV was increased from 50 to 200 ergs/mm^2. Treatment with a subeffective concentration (0.1 μg/ml) of MCA before or after the irradiation did not produce any transformed foci. Treatment with this concentration of MCA followed by TPA produced a significant number of type III transformed foci (table 2). Prior treatment with TPA followed by UV irradiation also did not produce transformation.

DISCUSSION

The experiments reported here clearly demonstrate that initiation and promotion stages of carcinogenesis occur in our C3H/10T1/2 mouse embryo cells in culture with UV light and TPA, just as we showed previously with carcinogenic hydrocarbons (*14*). In this system also, UV irradiation required the subsequent addition of TPA as a promoter for transformation. When we irradiated with UV alone, we failed to obtain type III foci at any dose level. Many dishes contained type I and type II foci, which we did not score as transformed because in previous studies, the cells from them seldom produced tumors when inoculated into syngeneic mice. The cells isolated from our type III foci produced tumors at high frequency at early passage. Recently, Chan and Little (*12*) reported the production of type III transformed foci in C3H/10T1/2 cells by UV irradiation alone. We do not know the reason for this difference. However, we do know that the passage number of the cells is an important factor in transformation experiments. Late passages (beyond 12) of C3H/10T1/2 cells sometimes show spontaneously type I and occasionally type II colonies. Therefore, we always use 4–10th passage cells for all our experiments, and our control dishes are always free from any type of focus. Chan and Little (*12*) used 11–15th passage cells for their experiments and gave no information about the appearance of their control dishes. If control dishes contained type II foci, it might be possible to obtain type III transformed colonies with UV alone. Moreover, in our experiments (table 2), repeated exposures to various doses of UV at various intervals did not produce type III transformed foci. This lack of transformation produced by UV light in the absence of TPA is in strong contrast to our results with polycyclic hydrocarbons, which produced transformation at 0.25–1.0 μg/ml but required TPA promotion when the hydrocarbon was given at a subthreshold concentration of 0.1 μg/ml (*14*). Thus in

C3H/10T1/2 cells and on mouse skin, polycyclic hydrocarbons act both as initiators and complete carcinogens, whereas in our experiments, UV acts as a pure initiator in C3H/10T1/2 cells. Moreover, treatment with 0.1 μg MCA/ml (an initiating dose) did not promote UV-initiated cells. Thus MCA does not possess pure promoting activity. Furthermore, treatment of the cells with TPA followed by UV irradiation or MCA led to no transformation [table 2; (*7, 14*)], which demonstrates that in these cells TPA acts only on initiated cells for oncogenesis.

The development of a reliable two-stage system enables us to perform critical studies (now underway) on the cellular and molecular mechanisms of initiation by chemical carcinogens and UV light and of promotion.

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Transformation Frequency of Syrian Golden Hamster Cells and Its Modulation by Ultraviolet Irradiation[1]

Joseph A. DiPaolo *and* Paul J. Donovan[2]

ABSTRACT—UV (253.7 nm) exposure of Syrian golden hamster cells seeded for colony formation caused an induction of transformation without any other insult. Analysis of the quantitative data demonstrated a lack of a threshold response and a transformation frequency proportional to UV dose. No transformation occurred in unirradiated colonies. When cells seeded for colony formation are exposed to UV and subsequently treated with AcAAF, a carcinogen known to induce UV-like damage, neither an additive nor a synergistic effect occurred. Caffeine at nontoxic concentrations potentiates lethality caused by AcAAF and also the number of colonies transformed by AcAAF when added post-AcAAF. The addition of a constant amount of caffeine (50 μg/ml) for 48 hours at different intervals after carcinogen resulted in maximum enhancement (10- to 17-fold) when added 4 hours post-AcAAF. UV-associated transformation was enhanced by pretreating the cells with a nontransforming dose of X-irradiation 48 hours before UV. The increase in transformation frequency was 6- to 12-fold per colony and 3- to 6-fold per dish.—Natl Cancer Inst Monogr 50: 75–80, 1978.

The use of in vitro models in which mammalian cells are used for studying transformation are a logical extension of the in vivo studies concerned with UV-associated carcinogenesis. With in vitro and in vivo studies, the end-point neoplasia can be demonstrated by tumor formation. Any in vitro system that is a step further removed from this specific end point loses its relevance to the cancer problem. In vitro systems with mammalian cells allow the study of target cell and nontarget cell interactions. A fundamental point to consider is whether transformation, first observed as an alteration in morphology, is a selective or an inductive phenomenon. The evidence obtained from the quantitative Syrian golden hamster cell model leads to the conclusion that transformation is an inductive phenomenon (*1*). The cells are negative for known viruses by the mouse antibody production and DNA–RNA hybridization tests (*2*). Furthermore, the tumors formed by transformed cells are immunologically distinct. Therefore, transformation is not due to viral activation. Statistical analysis of dose–response relationships proves that transformation is a one-hit phenomenon and the transformations have a Poisson distribution (Gart J, Donovan PJ, DiPaolo JA: Unpublished observations).

Much evidence has been obtained that UV is a causative agent for naturally occurring skin cancer. In addition, reports of several laboratory studies are available (*3*). Hueper (*4*) demonstrated in 1941 that with 253-nm UV treatment of rodents, epidermal sarcomas and carcinomas may result depending on the ratio of epidermis to connective tissue. In 1955, Rogers (*5*) showed that a massive dose of 253 nm UV to minced mouse lung produced cellular changes reflected in tumor formation when the cells were injected sc into mice. More recently, Setlow and Hart (*6*) exposed *Poccilia formosa* cells to UV and demonstrated that they underwent transformation. If the irradiation was followed by photoreactivation, the incidence of transformation was markedly reduced. It was concluded from these experiments that pyrimidine dimers induced by UV irradiation can lead to neoplastic transformation.

The action spectrum of nonionizing radiation for cancer is unknown. The specific wavelength, 254 nm, is absorbed before reaching earth and therefore is probably not important physiologically (*7*). However, this wavelength is near the maximum absorbance of DNA and is important from that point of view. There is evidence that this wavelength provides a model to study the phenomenon of mutagenesis and, in 1975, we reported (*8*) that this wavelength provides an in vitro model for the study of transformation. Subsequently, Mondal and Heidelberger (*9*) reported that UV was effective in transforming C3H mouse 10T1/2 cells only if followed by a promoting agent; however, Chan and Little (*10*), using mouse cells obtained from Heidelberger, claim that UV alone is capable of transforming these cells. Some of the experiments in which we used Syrian hamster cells are presented.

Cells derived from 12- to 14-day old Syrian hamster fetuses were grown as monolayers in Dulbecco's modification of Eagle's minimum essential medium with 10% fetal bovine serum in a humidified 10% CO_2 incubator at 37° C. For the quantitative assay, 300 cells/60-mm dish from secondary or tertiary hamster cultures were seeded on irradiated Syrian hamster feeder cells (*1*). UV irradiation at a dose rate of 0.76 ergs/mm^2/second or treatment with chemicals was done 24 hours later; incubation was continued for 6 to 7 days longer. The primary criterion of morphologic transformation, a lack of orientation and criss-crossing of cellular elements, was not observed in the controls. Two of the types of transformed colonies, contiguous and semicontiguous, were obtained after UV treatment (fig. 1). Transformed colonies can be isolated and subcultured and will form tumors when injected into hamsters or nude mice.

Dose–response experiments documented the relationship between transformation rate and UV dose (text-fig. 1). No

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Abbreviations: AcAAF = *N*-acetoxy-2-acetyl aminofluorene; PBS = phosphate-buffered saline; R = roentgens; BP = benzo[*a*]-pyrene; MNNG = *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; MNU = *N*-methyl-*N'*-nitrosourea.

[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] Biology Branch, Carcinogenesis Program, Division of Cancer Cause and Prevention, National Cancer Institute.

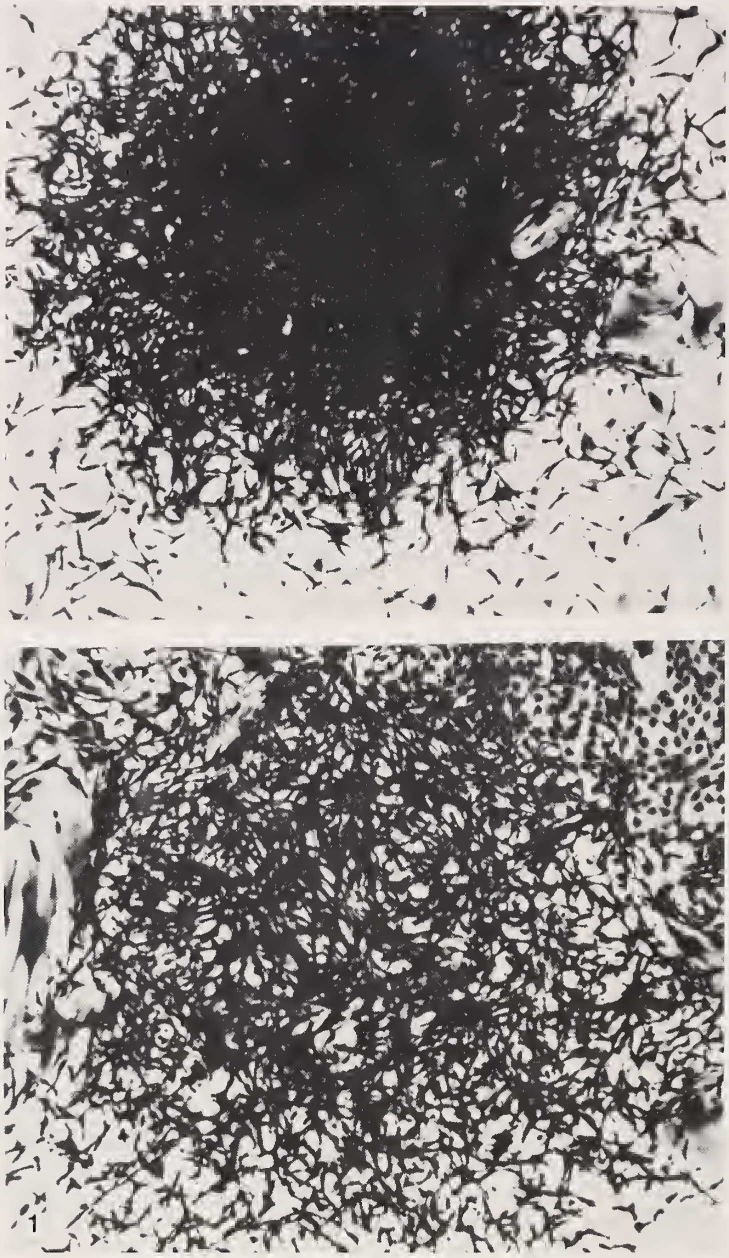


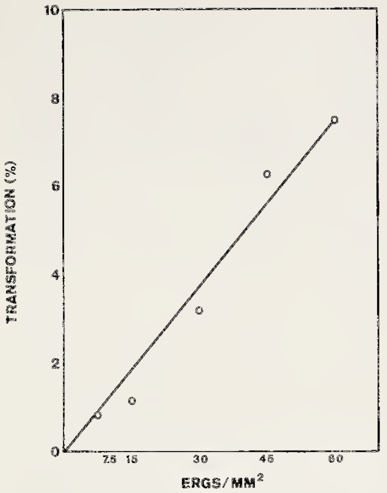
FIGURE 1.—Transformed colonies derived from cells of a secondary culture seeded to form colonies. Cells were exposed to UV (30 ergs/mm^2) 24 hr after seeded. Dense *(top)* and semicontiguous *(bottom)* colonies exhibiting transformation characterized as criss-crossing and loss of contact inhibition not observed in controls.

TABLE 1.—*Transformation of Syrian hamster cells by UV (253.7 nm)*

Ergs/mm^2	Total dishes	Transformed colonies	Transformed/ dish[a]	Cloning efficiency, %[b]	Transformed/ total colonies, % [a]
0	24	0	0	22.5	0
7.5	23	12	0.522	21.7	0.80
15.2	23	25	1.04	20.7	1.67
30.4	24	38	1.73	18.1	3.18
45.6	24	62	2.58	13.7	6.27
60.8	24	36	1.50	6.7	7.46

[a] For colony formation assay, 300 cells/60-mm dish were seeded; 24 hr later the medium was replaced with PBS while the cells were treated with UV; after irradiation, the PBS was replaced with fresh medium; incubation was continued for 7 more days before the colonies were fixed for examination. Average frequency of transformed colonies obtained per dish or total number of colonies examined.

[b] We determined cloning efficiency by dividing the average number of colonies per plate by the number of cells seeded per plate multiplied by 100.

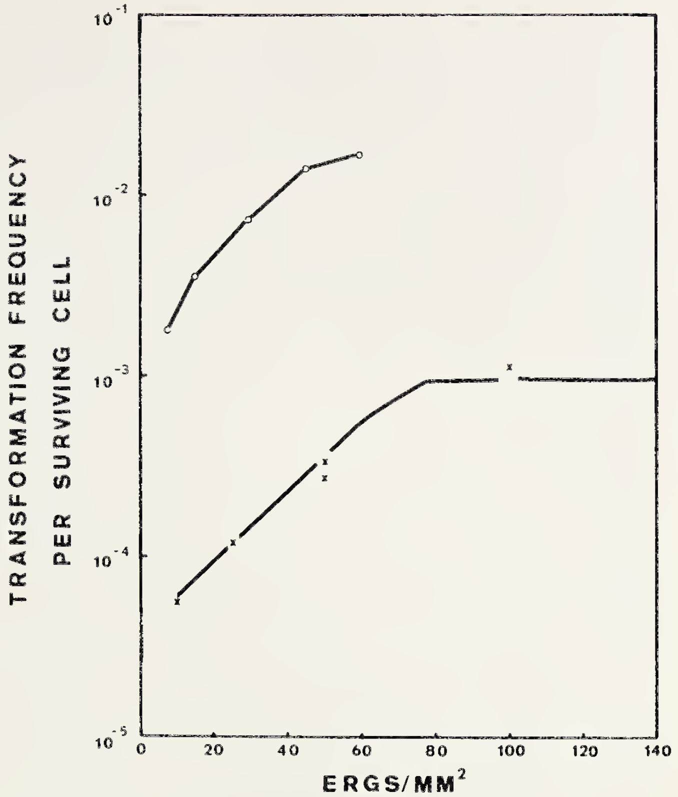


TEXT-FIGURE 1.—Transformation of hamster cells after exposure to various doses of UV radiation. Results expressed as transformed colonies/total colonies counted. × 100

evidence of a threshold response was observed nor was a transformed colony found without UV treatment. Increasing doses of UV raised the transformation frequency when calculated on the basis of transformed colonies per total surviving colonies. The best fit line extrapolated to the origin and thus demonstrated that transformation after UV was inductive rather than selective. However, transformation can occur without significant toxicity. In one particular experiment, the cloning efficiency of the control was 22.5% and, with a UV dose of 15 ergs/mm^2, although the cloning efficiency was 21.9%, a significant number of transformations were obtained. With high doses of UV, toxicity is increased and the absolute number of transformations decreased (table 1).

These results obtained with UV-irradiated Syrian hamster cells may be compared with those obtained with the mouse cell line 10T1/2 by Chan and Little (*10*). In text-figure 2, the transformation frequency per surviving cell for Syrian hamster cells and the mouse cell line have similar shapes, but the hamster cells are 50-fold more sensitive than the 10T1/2 cells. The plateau, which can be demonstrated more clearly with the 10T1/2, is an indication of the percentage of survivors. Transformation per cell, which can also be compared (text-fig. 3), is an absolute value and does not depend on relative survival of cells at different doses of UV. The two curves are similar with still a 50-fold difference between the two cell types.

When cells seeded to form colonies were treated first with UV and subsequently with AcAAF 6 minutes to 4 hours later, no significant enhancement of the transformation frequency occurred (table 2). The data are presented as transformations per dish and percentage of transformed colonies per total colonies. Analysis on the basis of the ratio of double treatment as well as the sum of the separate treatments demonstrated that the results are less than additive or not statistically significant.



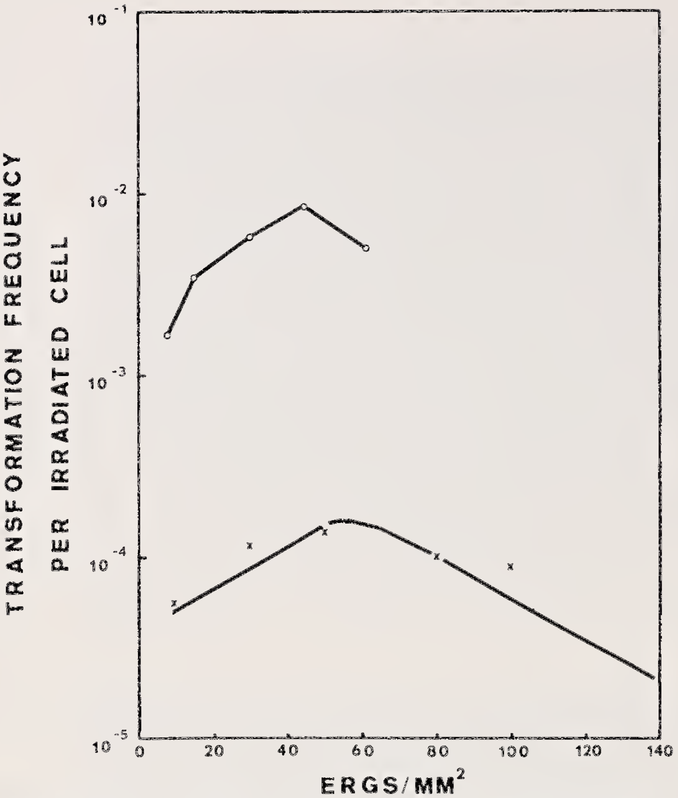
TEXT-FIGURE 2.—Transformation frequency per surviving cells as a function of UV dose. ○——○ = Syrian hamster fetal cells (*8*); x——x = 10T1/2 mouse fibroblast line (*10*).

If Syrian golden hamster cells were X-irradiated with 250 R, seeded for colony formation, and treated with UV irradiation at 15 ergs/mm^2, a significant enhancement of UV transformation occurred at 48 hours (table 3). The results parallel X-ray enhancement of BP transformation (*11*). Thus UV radiation mimics a chemical carcinogen.

Several hypotheses have been proposed to explain the interaction between X-ray and UV, but the mechanisms are inadequately understood. X-irradiation may affect the UV survival curve of bacteria but does not alter the final slope of the curves (*12*). The enhancement by X-ray of UV lethality may be due to the failure of the recovery process when damaging events are too numerous or too closely spaced to allow repair, or when the repair apparatus has been damaged to the point that it can no longer function.

UV-induced mutations have been demonstrated in mammalian cells including human cells. The mutation frequency is independent of the selecting agent and the cells used and is in the range 10^{-5} to 10^{-7}/erg/mm^2 (*13*). With freshly derived cells, the transformation frequency is 10^{-3}/erg/mm^2. This difference between mutation and transformation frequency approximates 10^2 to 10^4. Since it is accepted that mutation probably involves dominant structural genes, a high transformation frequency probably cannot be explained by the same mechanism. However, to be cautious, one could conservatively postulate that transformation could alter or could involve alterations of large blocks of material in the cell.

We have also been concerned with the role of chemical compounds (e.g., caffeine), which are known to potentiate cell killing by agents such as UV and alkylating compounds. We did a series of experiments using caffeine, a noncarcino-



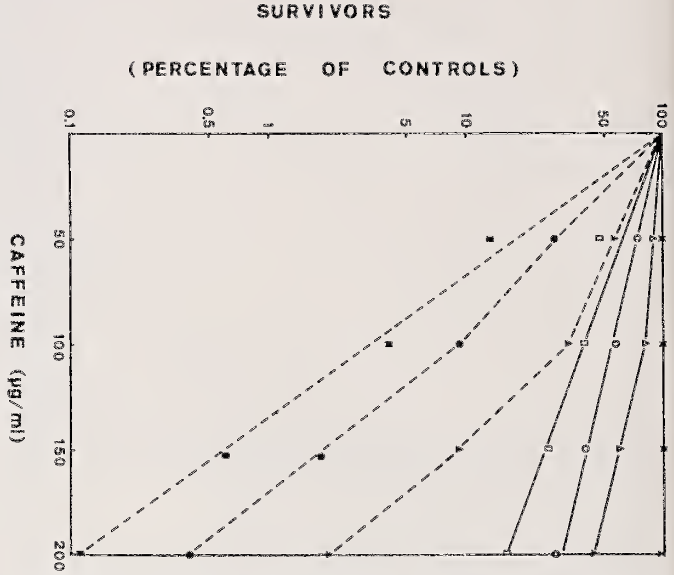
Text-figure 3.—Transformation frequency per irradiated cell as a function of UV dose. ○———○ = Syrian hamster fetal cells (*8*); x———x = 10T1/2 mouse fibroblast line (*10*).

gen, on Syrian hamster cells and demonstrated several conclusions (*14*). It is possible to increase the transformation frequency associated with a number of carcinogens by posttreatment with caffeine. The enhancement depends on a number of factors: It is influenced by the concentration and the time of addition of caffeine, the length of exposure to it, the specific carcinogen used, and the sequence of the carcinogen and caffeine treatment. The influence of caffeine on survival of cells pretreated with AcAAF for 1 hour and incubated 7 days resulted in the potentiation of toxicity relative to the untreated controls or cells treated with caffeine only. The percentage of cells capable of multiplying on hamster feeder layers was determined by the criterion of colony formation. Results were plotted according to the cloning efficiency obtained with untreated controls or cells treated with the carcinogens used: BP, AcAAF, and MNNG at 2.5, 1, and 0.25 µg/ml of medium, respectively (text-fig. 4). When caffeine, at concentrations of 50 to 200 µg/ml of medium, was incubated with cells for either 2 or 7 days, no toxicity was observed, i.e., the cloning efficiency obtained was identical to that for untreated controls (32%). Furthermore, no gross morphologic differences between caffeine-exposed and untreated control cells were observed with a phase contrast microscope.

Table 2.—*Transformation of Syrian golden hamster cells irradiated and treated with 0.5 µg AcAAF/ml*[a]

Treatment	Transformed cells/dish	Cloning efficiency, %	Transformed cells/ total colonies, %
UV	0.86	20.9	1.37
AcAAF	0.97	16.5	1.96
UV + AcAAF			
0.1 hr	1.08	9.6	3.74
1 "	1.25	10.1	4.14
2 "	1.54	12.4	4.13
3 "	1.25	11.0	3.79
4 "	0.67	10.6	2.11
Control	0	23.5	0

[a] After 24-hr growth of cells seeded for colony formation, the medium was removed and temporarily replaced with PBS during UV irradiation. PBS was replaced by complete medium and at the times indicated, AcAAF was added. Experiments were terminated on day 8. Transformation calculations were based on average number found in 12 dishes.



Text-figure 4.—Cloning efficiency (survival) of secondary hamster embryo cultures seeded to form colonies. Twenty-four hours after seeding, cells were treated with caffeine, carcinogen, or combination of carcinogen followed by caffeine 1 hr later. Incubation was for 2 or 7 days. When carcinogen and caffeine treatment was for 2 days, the medium was replaced with fresh medium for an additional 5 days. Results were normalized by consideration of 100% as the cloning efficiency of untreated controls and carcinogen only treatment. *Open symbols* on *solid lines* indicate 2-day exposures. *Closed symbols* on *dotted lines* indicate 7-day exposure. BP (2.5 µg/ml) △, ▲; MNNG (0.25 µg/ml) ○, ●; AcAAF (1.0 µg/ml) □, ■. Caffeine only for 7 days, x———x.

The addition of a constant amount of caffeine (50 µg/ml) for 48 hours at different intervals after AcAAF, a compound known to produce UV type of DNA damage, resulted in maximum enhancement (10- to 17-fold) when caffeine was added at 4 hours. Text-figure 5 shows 3 experiments with cell strains obtained from 3 hamsters. Addition of caffeine 24 or 48 hours after AcAAF had no effect; addition of increased concentrations of caffeine resulted in a reduced enhancement at 200 µg/ml, but when caffeine was added 1 hour pre-

TABLE 3.—*Transformation frequency in hamster cells X-irradiated 48 hr before treatment with 253.7 nm UV*

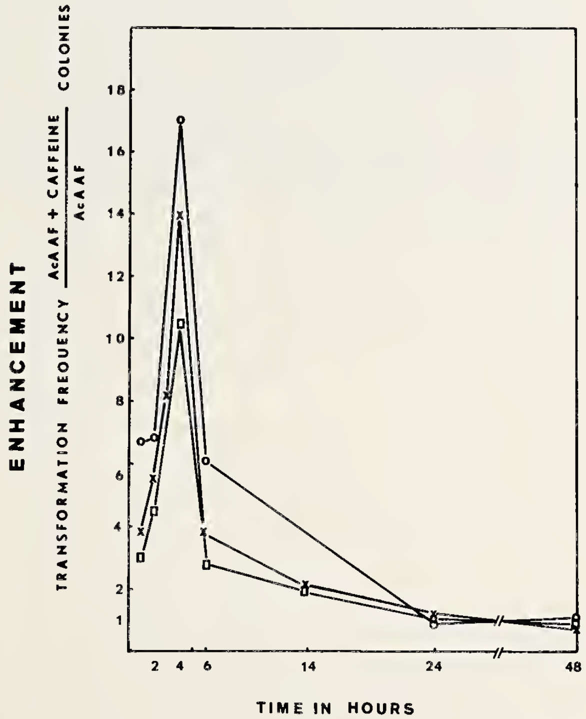
UV dose	Transformed colonies/ dish	Enhancement transformation/ dish[a]	Cloning efficiency %[b]	Transformed colonies: total colonies, %	Enhancement transformation/ colony
No X-ray					
0	0		24.7	0	
15	0.875	1.0	23.4	1.24	1.0
30	1.46	1.0	17.9	2.71	1.0
X-ray					
0	0		17.6	0	
15	5.57	6.36	12.3	15.09	12.2
30	4.13	2.83	9.1	15.13	5.57

[a] For X-irradiation enhancement of transformation, hamster cultures were exposed to 250 R, cloned, incubated for 48 hr; medium was temporarily replaced with PBS during UV irradiation and cultures further incubated for 7 days. Ratio of frequency = transformed colonies obtained with X-ray and UV to colonies obtained with UV only. The latter by definition is 1.

[b] We determined cloning efficiency by dividing the average number of colonies per plate by the number of cells seeded per plate multiplied by 100.

AcAAF, it did not affect the transformation frequency caused by AcAAF.

The complete data for one of the three curves are presented in table 4. The enhancement obtained by the addition of caffeine at 4 hours is independent of the potentiation of toxicity associated with AcAAF and caffeine. The relative cloning efficiency just before and after maximum enhancement of transformation are in the same range. Furthermore, the cloning efficiency at the time of maximum enhancement relative to that obtained with AcAAF is approximately one-third of that observed for AcAAF only. This provided additional evidence that the maximum enhancement in transformation frequency did not occur as a result of overwhelming lethality. Additionally, the absolute increase noted up to 4 hours after caffeine treatment in the average number of transformed colonies/dish is confirmation of the inductive characteristic of transformation.



TEXT-FIGURE 5.—Caffeine enhancement of transformation of hamster embryo cultures exposed to 1 μg AcAAF/ml medium 24 hr subsequent to cloning and followed by a 48-hr exposure to 50 μg caffeine/ml medium added at the times indicated. Number of transformed colonies from caffeine-treated cells was compared with those treated with AcAAF only and cultured for the same length of time. Results of 3 experiments are shown.

Caffeine has been reported to enhance simian adenovirus 7. Virus transformation enhancement also depended on the sequence of the addition of virus and caffeine (*15*). The effect of caffeine on mutation frequency in mammalian cells has also been studied (*16–18*). Depending on the caffeine concentrations and mutation technique used, caffeine depresses or enhances UV mutagenesis. Caffeine enhancement of MNU mutation induction occurred only with toxic concentrations of MNU. Chang et al. (*19*) found that caffeine added during the expression (time) for ouabain resistance will enhance mutation. If the caffeine is added for the entire selection period post-UV, the mutation frequency is decreased. Myhr and DiPaolo (*20*) have reported that, when using a selection procedure that allows maximum phenotypic expression of mutants, caffeine potentiated the AcAAF-induced lethality; the potentiation increased as the concentration of either caffeine or AcAAF was increased. Caffeine concentrations from 50 to 200 μg/ml had little effect on the mutant frequency induced by 0.1 to 2.5 μg AcAAF/ml. Caffeine can increase lethality without a concomitant increase in the frequency of induced mutants. Thus the mutagenesis results do not correlate with the 9- to 15-fold enhancement of AcAAF-induced transformation of Syrian hamster embryo cells caused by 50–100 μg caffeine/ml added 1 hour after 1 μg AcAAF/ml.

TABLE 4.—*Transformation of hamster fetal cells after AcAAF treatment followed by caffeine*

Caffeine post AcAAF, hr[a]	Cloning efficiency of AcAAF, %	Average transformed colonies/ dish	Enhancement/ dish[b]	Transformed colonies/ total colonies, %	Enhancement/ colonies[b]
—	100	0.78	1.0	1.12	1.0
1	64.1	1.91	2.5	4.28	3.8
3	34.9	2.22	2.9	9.14	8.2
4	33.0	3.58	4.6	15.58	13.9
6	38.7	1.14	1.5	4.22	3.8
14	77.5	1.85	1.6	3.05	2.1
24	97.9	0.94	1.2	1.19	1.2

[a] We seeded 300 cells (60-mm dish) for colony formation; 24 hr later the cells were treated with 1 μg AcAAF followed by 50 μg caffeine/ml medium at the times indicated; 48 hr after addition of caffeine, the medium was replaced by fresh medium without caffeine; corresponding dishes with AcAAF only (—) were also refed.

[b] Ratio of frequency of transformed colonies was obtained with AcAAF and caffeine to colonies obtained with AcAAF only; the latter by definition is 1.

The repair of DNA damage caused by the chemicals used in this study is known to be of the UV type. In the final analysis, the type of lesion responsible for lethality or mutation whether in the DNA or DNA–protein complexes is not known; in fact, Cerrutti (*21*) postulated that certain DNA-damaging agents may be potent mutagens but weak carcinogens.

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Discussion: Characteristics of Ultraviolet-Induced Tumors[1]

M. Hanna: We can now open these talks for discussion.

J. Epstein: My comment is directed to Dr. Stenbäck. It has been our experience with the production of UV carcinomas in mice that we get primarily the premalignant and malignant changes, and we rarely get keratoacanthomas. Did I misinterpret what you said earlier, or is your experience different from ours?

F. Stenbäck: The incidence of UV-induced keratoacanthomas in experimental studies depends on the materials and methods used: animal species, strain, light source, total dose as well as intensity of irradiation. In our published work, we used mainly Swiss mice but also rats and Syrian golden hamsters. In a short period, they were given a fairly intensive exposure, which induced initial injury with subsequent keratoacanthoma formation. UV irradiation in smaller doses over extended periods is mainly associated with squamous cell carcinoma formation.

R. Freeman: Dr. Stenbäck, I was particularly interested in your reference to the production of basal cell carcinomas with UV. I wonder if you could give us a few more details about the conditions of their occurrence. I spent 2 or 3 years attempting to produce basal cell tumors with UV and was unsuccessful. They have been produced with ionizing radiation, but I am not sure that anyone has produced them experimentally with UV.

Stenbäck: Basal cell carcinomas are easily induced in rats by polycyclic hydrocarbons such as 20-methylcholanthrene and 7,12-dimethylbenz[*a*]anthracene. In UV-irradiated animals, we have frequently seen follicular changes but rarely hair follicle tumors, and the incidence of basal cell carcinomas is below 2%. As shown here, basal cell carcinomas are of different types, but the total number is low. Our efforts to develop a model that consistently produces a high incidence of basal cell carcinomas with UV light has not been very successful.

F. Urbach: There has to be a significant amount of radiation damage to the skin in the way of atrophy and dermal changes (which could be lumped grossly under the heading of radiodermatitis) before even the earliest tumors of the solar keratosis–squamous cell carcinoma axis appear. I think that is a most important point.

As far as basal cell carcinoma is concerned, I again agree. One-third of these certainly do not seem to be related to light. Another interesting point about basal cell carcinomas that apparently is not genetic has to do with body area. If one excludes things like multicentric basal cell carcinoma (clearly a different variety), most nodular basal cell carcinomas occur on the head and neck. The reason for that is totally unclear to me. The same is true of X-ray-induced skin cancer on the head and neck: About 50% of the tumors seem to be basal cell carcinomas. Most X-ray-induced skin tumors located elsewhere on the body are squamous cell carcinomas; only a few are basal cell carcinomas, and occasionally melanomas, lymphosarcomas, and sarcomas are seen.

Some peculiar substrate on the head and neck allows, under various stimuli of which UV light is apparently one of the major factors, the development of basal cell carcinomas in that particular area, and thus there is that regional factor.

W. Clark: I do not know the histogenetic factors responsible for the dominance of basal cell carcinoma on the head and neck. The point that I have been trying to make is that the term "basal cell carcinoma" includes a variety of tumors, many differentiating along skin appendage lines. If one were to study the histology of basal cell carcinoma occurring in areas of greatest light dose and damage, one could delineate those forms of basal cell carcinoma that are light induced. One could envisage the delineation of 3 classes of basal cell carcinoma: 1) light induced, 2) light related, and 3) light independent.

J. DiPaolo: The discussion of the two in vitro papers is open.

T. Coohill: I have a question for Dr. Mondal. First of all, I agree with you that as cells "age," as defined by passage number, their radiation sensitivity may change. We work with African green monkey kidney cells, and we find that we have to limit the number of times we can split them to get consistent results. You used the words "spontaneous transformation." Is it not true that if it is really spontaneous transformation, Dr. Little would find the same thing in his controls as he does with his UV irradiation?

S. Mondal: I have seen some of his control dishes, and some of them had types I and II foci.

DiPaolo: Is it true that your type I is considered not to be a transformed focus but some kind of a background noise?

Mondal: Type I is not a transformed colony.

DiPaolo: I have a copy of the paper by Chen and Little, and they scored only type III transformations as you also did. These were not found in the controls. If I can take the prerogative of the Chairman, I would like to limit the questions to those that pertain to the presentations of the people here. Some, such as Dr. Pienta, have done in vitro work. Dr. Pienta, would you care to comment? If not, I would like to call on Dr. Smith, please.

K. Smith: I have comments on Dr. DiPaolo's paper. From studies on mutagenesis in bacteria, the number of mutants per plate reaches a maximum at about the D_{37} UV dose. Does this dose correlate with the enhancement of transformation that you saw as a function of dose?

DiPaolo: Our D_{37} was 58 ergs. The enhancement results were obtained even with 15 ergs, which is much, much below the D_{37}. As I indicated, minimal toxicity occurs with 15 ergs.

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[1] Conducted at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

Smith: The other comment was that you spoke about interactions of X-ray and UV, but you were referring to experiments in which the UV and the X-rays were given simultaneously. In more recent work, with a lag between the X-ray and the UV irradiations, the bacterial cells were much more resistant to the subsequent UV irradiation. Thus you get synergism (enhanced killing) if you give UV and X-ray at time zero, but you get antagonism (reduced killing) if you incubate between the two doses.

E. De Fabo: Dr. Mondal, I may have missed this in your talk. What was your source of UV? Was it a germicidal lamp and what was the dose rate?

Mondal: It was a germicidal lamp. We started from 10 ergs/mm^2, and we went up to a total dose of 200 ergs/mm^2.

De Fabo: What was your initial dose rate?

Mondal: That varied from time to time, but we have an ergometer with which we always calculate the dose before irradiating.

SESSION III

Production and Measurement of Ultraviolet Light

Chairman: I. Magnus

The Biologic Effectiveness of Ultraviolet Light[1, 2]

Claud S. Rupert[3]

ABSTRACT—The biologic effects of visible and UV light result from photochemical changes in cell components. The amount of photochemical change induced in a small non-self-shadowing structure is proportional to the number of photons traversing it per unit area normal to the direction of propagation, summed over all component beam directions. Within an optically complex, absorbing, and scattering structure, this quantity is difficult to determine, but for skin it is approximately proportional to the total number of photons per unit area entering its outer surface. The magnitude of some photobiologic effects depends on the total amount of photochemical change induced, whereas others depend on the rate of photoproduct formation or on a more complex relation. The nature of the dependence must be determined before light measurements can be related to the magnitude. The effect of a polychromatic illumination depends on its wavelength distribution, weighted by the effectiveness of each wavelength (the action spectrum) under the conditions employed. Until the latter is known, no dosimetric characterization of the light is possible. The wavelength distribution can be determined by spectroradiometric measurement, with the weighting performed numerically, or (more conveniently, though less accurately) by the use of an analog reaction with an action spectrum like that of the photobiologic effect.—Natl Cancer Inst Monogr 50: 85–89, 1978.

Production and measurement of UV light belong to applied physics and engineering, but the features of light that are relevant to UV carcinogenesis, i.e., what we wish to measure and why we wish to measure it, depend on the photobiologic phenomenon itself. We begin by examining the rationale behind photobiologic dosimetry.

All effects of light on a living thing, whether normal processes like vision or pathologic responses like sunburn and carcinogenesis, stem from photochemical changes produced in some of its components. Therefore, the initiating event is always the absorption of a photon in a molecular structure, putting it into an excited state from which the molecule can undergo an otherwise inaccessible reaction. Although a complex sequence of biochemical and physiologic events leads from this photoproduct to the final effect, the photochemical nature of the initial step stamps its imprint on the induction process. This characteristic affects the light measurements required.

PHOTOCHEMICAL EFFECTIVENESS OF LIGHT

Suppose that some number of photoreactive molecules, e.g., dN, is present within an elementary volume dv (which we might suppose has a broadside area dA and depth dl, as in text-figure 1: dv=dA · dl). Furthermore, suppose that this volume is irradiated with a monochromatic light beam until F photons per unit area normal to the beam direction have traversed the region containing dv. If the molecular cross-section for absorption (i.e., the effective "target size" for intercepting a photon) is σ,[4] then the total number dn, which is raised to an excited state, will be equal to the number of photons which pass through dv times the likelihood that each photon encounters an "effective target" on the way: i.e.,

$$dn = (F\,da)\left[[\frac{\sigma dN}{dA}\right] = \sigma\ F\ dN$$

Some fraction ϕ of these excited molecules will undergo the photochemical reaction in question (ϕ being the quantum yield). Consequently, the fraction of the molecules present which will be altered is

$$f = \phi\ \frac{dn}{dN} = \sigma\ \phi\ F. \tag{1}$$

The values of σ and ϕ depend only on the molecules absorbing the light and on the conditions in their immediate surroundings; F represents the light treatment applied to them.

The quantity measured by F does not have an agreed-upon name in photochemistry or photobiology at the present time. The analogous quantity for ionizing radiations is known as "fluence," but adoption of this term is opposed by some on grounds that its Latin root suggests a flow rate, rather than the time integral of a flow. When it is necessary to have a name, we use this one for lack of any other, without necessarily implying advocacy.

The relationship between the fraction f of the original molecules altered (or between the total number of such altered molecules, n = f·N), and the observed effect depends on the biologic details. The UV inactivation of cells and viruses under certain commonly encountered conditions depends only on the total amount of photoproduct generated, as shown by the fact that the consequences of irradiation with the same fluence, delivered at different intensities for different lengths of time, are similar. However, this is not true for all photobiologic effects. The visual system involves dark and light reactions, which combine to make the time *rate* of photoproduct creation the important factor; for some other phenomena, the dependence on light treatment is more complex. The nature of the dependence on F can be determined

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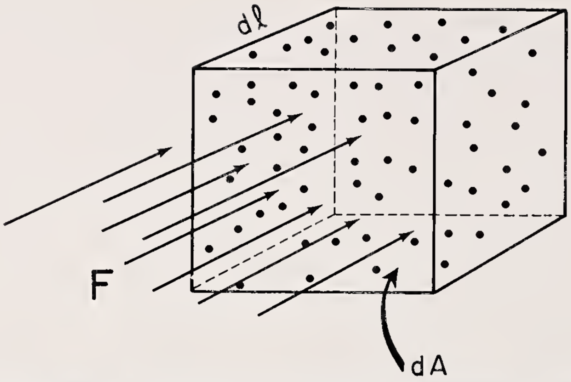
[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] Supported by Public Health Service Research Grant 2R01-GM16547 from the National Institute of General Medical Sciences.

[3] Programs in Biology, The University of Texas at Dallas, P.O. Box 688, Richardson, Texas 75080.

[4] The absorption cross-section of a molecular structure is related to the molar absorption coefficient ϵ by $\sigma[\mathrm{cm}^2] = 3.83 \times 10^{-21}\ \epsilon$ [liter·mol^{-1} · cm^{-1}].



TEXT-FIGURE 1.—Schematic irradiation of an elementary volume containing photoreactive molecules.

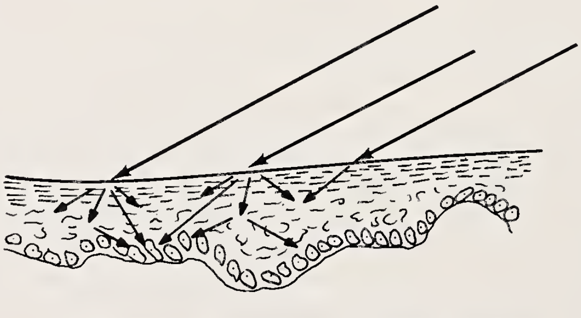
only by experimental study, and for UV carcinogenesis, it has yet to be fully determined.

If the irradiation comes from a number of different directions, F is the sum of the fluences contributed by each component beam direction. This quantity is, therefore, not in general equal to the energy per unit area entering any one particular surface, despite its dimensions (energy)/(area). It can be described as the energy entering a small sphere divided by the cross-sectional area of the sphere.

ILLUMINATION IN VIVO

In an intact animal, determination of the fluence at the site of the photosensitive molecules is usually a difficult matter. Consider for example the skin, for which a simplified sketch is shown in text-figure 2. UV light impinging on its outer surface is partly absorbed (in a wavelength-dependent manner) and strongly scattered during its passage through the optically nonhomogeneous corneum. By the time it reaches living cells, UV light is aimed in many directions unrelated to its original orientation, something like the daylight arriving at ground level on a hazy or overcast day. It continues to be scattered and absorbed with passage through successive cell layers. Although the magnitude of the fluence, on which the photochemistry depends, is hard to calculate, it is approximately proportional to the energy per unit area entering the outer skin surface at any specified wavelength. This latter quantity has long been defined as the *dose* of UV radiation (*1*).

For a given light beam and exposure time, the UV dose so defined is proportional to the cosine of the angle of incidence, measured from the normal to the skin surface. Such a dependence evidently constitutes the reason why skin areas oriented more nearly broadside to the sun during normal activities (nose, lips, upper cheeks, etc.) are the first to sunburn in casual sunlight exposure and also why they may show the highest incidence of nonmelanoma skin cancer (*2*). In situations in which the subject or the experimental animal can move at will, habits and preferences in orientation toward an UV source will be important.



TEXT-FIGURE 2.—Schematic irradiation of skin.

The dose is a highly practical quantity for characterizing UV treatment of skin. Even though it does not directly indicate the photochemical potency of the light treatment, the dose describes the experimental parameter which the investigator has under his control and is the reasonable quantity to report in connection with experiments.

POLYCHROMATIC DOSIMETRY

Because the product of absorption cross-section σ and quantum yield ϕ depends on the wavelength, photons of different energies show different effectiveness for inducing the initial photoproduct. When only a single photochemical process is involved, this fact plus any wavelength-dependent filtering in the organism are responsible for the whole effect of changing the wavelength. Under such circumstances, irradiation by several different wavelengths at once gives simply the sum of the effects produced by each one individually. However, photobiologic phenomena are known in which two or more photoprocesses influence the final effect, and when this occurs, the different phenomena must be dissected before summing the contributions the various wavelengths make to each. One may or may not have the information to do this effectively. The remarks apply, of course, to light with a continuous spectrum like natural daylight.

The assumption that only a single photoprocess causes a particular effect is a hopeful and not unreasonable first approach, but by no means is it assured until specifically investigated. When it is true, the contributions according to equation 1 for each small wavelength range

$$df = \sigma(\lambda)\ \phi(\lambda)\ dF(\lambda)$$

integrate to give

$$f = \int \sigma(\lambda)\ \phi(\lambda)\ (dF/d\lambda)\ d\lambda. \qquad (2)$$

One can put this in the same form as equation 1 by choosing a reference wavelength λ_r at which the cross-section and quantum yields have values σ_r and ϕ_r, respectively, and then writing the product of their values at any wavelength as

$$\sigma(\lambda)\phi(\lambda) = \sigma_r\phi_r A(\lambda). \qquad (3)$$

$A(\lambda)$, the relative action spectrum, is evidently equal to 1 for $\lambda = \lambda_r$. Inserting this in equation 2 gives

$$f = \sigma_r\phi_r <F> \qquad (4)$$

with

$$<F> = \int A(\lambda)(dF/d\lambda)d\lambda. \qquad (5)$$

Equation 4 differs from equation 1 in one important respect: Whereas F in equation 1 depends only on the light source and how it is used, the effective (or action-weighted) fluence $<F>$, defined by equation 5, depends also on properties of the system being irradiated, as summarized by A(λ).

It is impossible to describe a quantitative measure for polychromatic light treatment without first agreeing on the action spectrum. If $A(\lambda)$ is constant over the wavelengths where $dF/d\lambda$ is appreciably different from 0, equation 5 reduces to equation 1.

Everything we have just said is also true for the UV dose on the skin surface because of the direct proportionality between this quantity and the fluence inside the skin at the site of the affected cells. However, an additional wavelength-dependent filtering factor, due to scattering and absorption in the outer layers, will modify the shape of observed action spectrum.

THE ACTION SPECTRUM

Any wavelength in the effective range might be chosen as the reference λ_r for the action spectrum, but a selection must be made before the value of the polychromatic "effective fluence" $<F>$ is fixed. A common and usually convenient choice is the wavelength for which $A(\lambda)$ has its maximum value. With this choice, A is always less than or equal to unity. However, when the maximum value falls outside the wavelength region where the light source of interest has appreciable intensity, other choices may be preferred. This is often done for the sunburn action spectrum with a reference wavelength near 300 nm.

The relative action spectrum is commonly determined by a comparison of the effects of different wavelengths taken one at a time. In doing this, it is presumed that the different wavelengths are all doing exactly the same thing but with different efficiencies, a proposition which implies that the dependence of the effect on either dose or fluence is qualitatively the same at each wavelength. If such is true and the effect is the same function of dose over some appreciable range of dose rates, then $A(\lambda)$ will be inversely proportional to the dose required for the same magnitude effect at each λ. The relative values so determined can be normalized to the value at the reference wavelength taken as unity. A complete determination would also check whether illumination with a combination of wavelengths gave the expected simple summation of the effects of each separately. If this is not so, the assumptions underlying equation 2 do not hold, and equations 4 and 5 cannot be expected to describe the effects of polychromatic illumination. However, such a check is not often made because of the extra effort and experimental trouble required, particularly for effects which entail time-consuming exposures.

With any other simple dependence on the light treatment (e.g., an effect proportional to dose rate) one could also readily assess the relative effectiveness of different wavelengths, but with a complex dependence (one involving dose rates and exposure regimens as well as the total dose), more care would be necessary. Such a dependence would not correspond to the simple consequence of a single photochemical reaction, and the proper procedure would depend on the use intended for the action spectrum once it was determined. If it were to be used for estimating the effect of a polychromatic dose applied according to some specific regimen, it would seem best to measure the effectiveness of radiation at each single wavelength with that same dose regimen.

These considerations aside, it should be recognized that UV radiation injury to an organ as complex as skin is inherently more complicated than the model we used for discussion. The incident UV radiation is attenuated as it passes through successive cell layers and causes appreciable injury in successively deeper (and therefore differently differentiated) cells as the dose increases. Moreover, the attenuation is not the same for different wavelengths, increasing exponentially with decreasing wavelength in the 300-nm region. Therefore, the same dose given at different wavelengths may cause a qualitatively different pattern of damage in the array of cells comprising the tissues, even if the same photochemical events occur in each cell. This creates a problem crudely like that of asking how much surface abrasion is equivalent to a deep bruise. The answer necessarily depends on the criteria by which one judges "equivalence." Consequently, it is not surprising that the shape of the erythemal action spectrum changes when different "end points" are used to equate the effects of UV doses applied at different wavelengths [*see* (*3*)].

DAYLIGHT ILLUMINATION

$A(\lambda)$ for many UV effects on organisms rises steeply with decreasing wavelength through the 300-nm region, about an order of magnitude for each 3 nm of wavelength change, as would be expected if nucleic acids, proteins, or both, are primary absorbers of the light. The spectral distribution of natural daylight, on the other hand, *decreases* rapidly with decreasing wavelength in the same region. The combination could hardly be more inconvenient for estimating the biologic effects of solar UV, for it means that both $A(\lambda)$ and $dF/d\lambda$ at the photochemical site change rapidly over the only wavelengths at which their product contributes appreciably to $<F>$. Under these circumstances, $<F>$ is inordinately sensitive to small changes in $dF/d\lambda$ at wavelengths for which $A(\lambda)$ is highest. $<F>$ is also affected by changes in $A(\lambda)$ that do not greatly alter its appearance to the eye, because its values at different wavelengths are so differently weighted by $dF/d\lambda$. The result makes problems both for defining the biologically effective solar UV dose to the skin and for measuring it once it has been defined.[5]

Like everything else measured experimentally, action spectra are imperfectly known, and successively better determinations will serve to redefine the action-weighted dosimetric quantities relating to any particular effect. Since not every redetermination of an experimental quantity is an improvement, an "official" action spectrum, with provision for regular review and updating, should be designated by a suitable standards group.

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[5] Other shapes are, of course, possible for UV action spectra. A UV photoeffect caused by a sensitizing pigment will have the wavelength dependence of $A(\lambda)$ dictated by σ for the absorbing substance and ϕ for the photochemical reaction, as modified by any in vivo filtering. Such a product may well have its maximum at a wavelength removed from the ∾300-nm border of the daylight spectrum.

SPECTRAL DISTRIBUTION OF LIGHT

Given an agreed-upon action spectrum, the measurement of spectral distribution for either the fluence or the dose delivered by a polychromatic source and its spectral weighting ("convolution with the action spectrum", as in equation 5), are obtained by essentially two different kinds of procedures. The simplest methods involve analog computation of the spectral weighting. The Robertson-Berger meter (*4*), a recent, highly successful example, uses the fluorescence of magnesium tungstate, which has an excitation spectrum resembling that of erythemal action. Another method is provided by photochemical reactions having approximately the desired action spectra, either producing colored products from colorless precursors (*5*) or spectrophotometrically observable changes in the precursor (*6*). Such a system can be embedded in plastic films, permitting convenient placement and easy readout of accumulated exposures. Even the inactivation of microorganisms under suitable controlled conditions has been used for dosimetric measurement (*7*). All these procedures have the advantages of simplicity and direct accessibility of the resulting measurement without elaborate calculations, but the desirable features are also linked to their disadvantage; being only analogs of the photobiologic processes under study, their action spectra cannot be "fine-tuned" to the desired form. They simply are whatever they are. The Robertson-Berger meter gives a lower relative weight to the shortest wavelengths in natural daylight than does the erythemal or the germicidal action spectrum (*4, 8*). Consequently, as the proportion of shorter wavelengths changes with variations in solar altitude or in the amount of stratospheric ozone, the instrumental calibration changes. This limitation is well recognized by the inventors and the knowledgeable users of such devices, who choose their tasks and interpret their data accordingly. However, simple devices readily get into unsophisticated hands. We still find people using meters that respond with the spectral sensitivity of the human eye to measure light for photobiologic phenomena with entirely different action spectra.

A more refined, but usually slower and more complicated and expensive, procedure is to use a spectroradiometer to characterize the illumination over the whole effective wavelength range and to apply the spectral weighting to these data numerically. This method gives the experimenter complete freedom to choose whatever action spectrum is presumed best, to explore the effects of different possible action spectra, or to correct old data on the basis of new and better action spectrum information. It also imposes a salutary necessity to think about what action spectrum ought to be used. A spectroradiometer is not a particularly simple instrument. It must provide means for sampling the light to which the organism is subjected and for doing absolute radiometry at prescribed wavelengths with a known, narrow spectral bandpass and a known instrumental transmission. Accomplishing this with reasonable speed and accuracy, both in measurement and calibration, is a matter of instrumental design and practical engineering. Such achievements are usually influenced heavily by user demand; i.e., the state of the art follows the state of the market. With the increasing incorporation of electronic microprocessors into instruments and the consequent increased speed of data acquisition and data reduction, one may hope for convenient devices, although the cost will probably remain high.

UV SOURCES

Although we are interested in the effects of solar UV radiation, the sun itself is a terrible source for experimental purposes. It varies in intensity and spectral distribution throughout the day (even when not interrupted by clouds), besides varying widely day by day and seasonally (*9*). Except for actual solar monitoring or for rough experiments, an artificial light source is required.

The source with which most UV work has been done, i.e., the low pressure mercury arc or "germicidal lamp" (which emits primarily the single mercury resonance line at 253.7 nm), is simple, stable, and monochromatic, yet not particularly suitable for simulating solar irradiation of animals. The photobiochemical effects of this radiation are similar to those of 300-nm UV, but its attenuation in traversing the skin is more than an order of magnitude greater. This means that irradiation of the outer layers must be heavy before lower levels in the epidermis receive much UV. Not only is the balance of irradiation from one cell layer to the next entirely different than in sunlight, producing a different ratio of shallow to deep injury, but the possibilities for production of photoproducts from extracellular materials in the heavily irradiated outer layers is increased, with a greater chance of actually introducing different kinds of phenomena. Although some excellent earlier work has been done with this source, at this time it should not be considered the prime choice on intact animals.

In some ways, a strong monochromatic source emitting near 300 nm would be the most desirable substitute, since single-wavelength irradiation bypasses all questions of action spectra in comparing work at various times and from different laboratories. The cluster of spectral lines near 313 nm in a high pressure mercury arc offer a possibility, although good interference filters would be required to separate these from other strong simultaneous emissions. This, plus the fact that more than an order of magnitude greater fluences are required for photochemical effects in proteins and nucleic acids at 313 nm, compared with 254 nm, complicates the set-up, compared with the germicidal lamp, which emits essentially one single, highly effective wavelength.

Combinations of continuous-spectrum lamps with filters have been developed for simulating increased solar UV effects on plants at various reduced levels of atmospheric ozone (*10, 11*). Westinghouse FS-40 Sun Lamps with layers of Kodacel TA-401 polyester, for example, give a good simulation of the short wavelength profile of the daylight spectrum between 320 nm and the cutoff around 290 nm. However, they are unlike daylight at longer wavelengths. The polyester filters change their transmission with exposure to the lamp radiation and so require regular monitoring and replacement, but these sources are simple enough to be used for supplementary irradiation of plants outdoors. Combinations of high intensity xenon lamps with glass filters have also been used to simulate biologically effective daylight UV in a more complex set-up indoors. The glass filters have an advantage in not changing with age. Although these sources are not claimed to be ideal, they are workable and provide models for further development.

RELEVANCE OF DOSIMETRIC QUANTITIES

Predicting the photobiologic effects of any polychromatic source requires dosimetry of its radiation over the effective wavelength range (either by spectroradiometry and calculation from an explicit action spectrum or by use of a suitable analog device), and comparison of experiments by different workers cannot be better than the quality of these data. On the other hand, emphasis on careful measurement sometimes fosters the impression that good measurements will necessarily be significant. This is true only if the photobiologic process in question is sufficiently well understood. In UV carcinogenesis, the dependence on UV-exposure is partly known but is evidently far from simple (*12*). The quantitative dependence is particularly unclear for human melanoma (*13*). Although further progress can come only by proceeding on the basis of our partial knowledge, a certain wary skepticism should be maintained about the predictive power of UV dosimetry until the carcinogenic process is better characterized.

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Workshop on Production and Measurement of Ultraviolet Light: Light Sources for Solar Simulation in Photocarcinogenesis Studies[1, 2]

P. Donald Forbes[3]

Outside the earth's atmosphere, sunlight contains a substantial amount of UVR, energy in the spectral region below λ 400 nm. The earth's atmosphere, especially its ozone content, is an effective filter, transmitting longer than 320 nm freely, but absorbing virtually all photons with wavelengths less than 295 nm. A plot of energy versus wavelength of two solar spectra, one on earth and the other above the atmosphere, would look qualitatively like text-figure 1. In fact, this figure does not represent actual sunlight but was made from a laboratory light source discussed below. Absolute energy units (*y*-axis) would apply only to the laboratory source.

The spectral quality of sunlight is not constant, and one significant factor in the variability is the effective thickness of the atmosphere through which the light passes. Effective thickness in turn is a function of such factors as the geographic location of the observer (latitude, altitude) and position of the sun in the sky (zenith angle). The influence of effective thickness can be modeled in the laboratory by the passing of light from a solar simulator (xenon arc lamp) through various thicknesses of ozone gas; this modeling is based on the observations that the xenon arc emission in the UVR and visible region is qualitatively similar to that of the sun (*1*) and that ozone is the principal absorber of UVR in the atmosphere (*2*). The influence of effective thickness (nominal ozone path) is illustrated in the plot of energy per unit area versus wavelength (text-fig. 2).

Each curve represents a source spectrum generated by directing the radiation from a long-arc xenon arc lamp through a gas flow chamber containing a known concentration of ozone in oxygen. The data were recorded by a Cary Model 14 spectrophotometer (Varian Associates, Palo Alto, Calif.) with radiometer accessory (*3*). Ozone itself is not yet a practical optical filter for routine application because it requires elaborate generating, handling, and measurement techniques.

The long-arc solar simulator that we use (Model RM-60, Atlas Electric Co., Chicago, Ill.) can be fitted conveniently with one of three types of inherent filtration that transmit sunburning UVR (UV-B; λ <320 nm). The three are borosilicate (Pyrex), IR-absorbing, and quartz glass. The borosilicate filter and the IR-absorbing filter both attenuate the UV-B to some extent, and both show some evidence of solarization with use. In text-figure 3, a new borosilicate filter (upper curve) and a used one are compared.

The IR-absorbing filter cuts off sharply in the UV-B. New and used filters are shown in text-figure 4.

We used this type of filter for several years in projects requiring simulated sunlight because it represented the best technology available for irradiating large areas (*4, 5*).

The third type of inherent filter material is quartz glass. This material serves well as the required jacket for the cooling water system (the primary purpose of the inherent filter), but it does not attenuate UV-B. The light quality with this filter in use is shown in the upper curve of text-figure 1. For this light source to be useful, additional filtration must be provided. The lower curve of text-figure 1 shows the result of inserting a Schott WG 320 (2 mm thick) filter in the beam.

Schott glass WG filters (Fish Shurman Co., New Rochelle, N.Y.) have several advantages in this application; one is that they do not solarize during several hundred hours of use. The second advantage is their availability in a range of thicknesses with a resulting family of spectral curves (text-fig. 5).

Third, and perhaps most important, is the similarity of the spectral curves produced by filtration through WG 320 or ozone. In text-figure 6, a 1-mm thick WG 320 filter (heavy line) is compared with 2.12 and 2.72 mm of ozone.

We consider plastic filter materials unsuitable for our purposes because of their tendency to solarize and their limited availability in multiple reproducible thicknesses. A direct comparison of the available glass filter types is reiterated on linear coordinates in text-figure 7. Curves 2 and 3 show, respectively, new and used borosilicate glass inherent filters; curves 4 and 5 show new and used IR-absorbing glass filters; curve 6 is the result of quartz glass inherent filtration plus WG 320 (2 mm) added filtration.

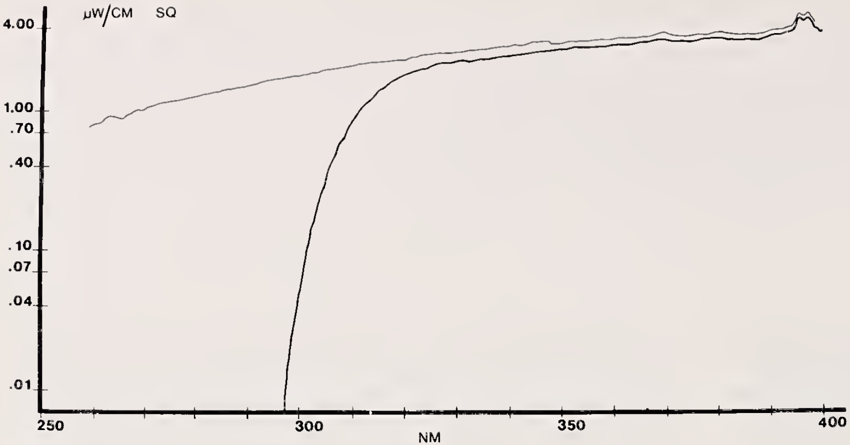
Part of the reason for wanting the best available match to solar spectrum is the uncertainty about action spectra of biologic responses. There is general agreement on only a part of the erythema action spectrum (*6*). Considerably less is known directly about the photocarcinogenesis action spectrum. Therefore, we believe that questions about light interaction can be investigated most appropriately through application of defined, solar-matched spectra.

Abbreviations: λ = wavelength(s); UVR = ultraviolet radiation; UV-B = 280–320 nm; STP = standard temperature and pressure; SUV = sunburning ultraviolet (meter); R-B = Robertson-Berger (sunburning meter).

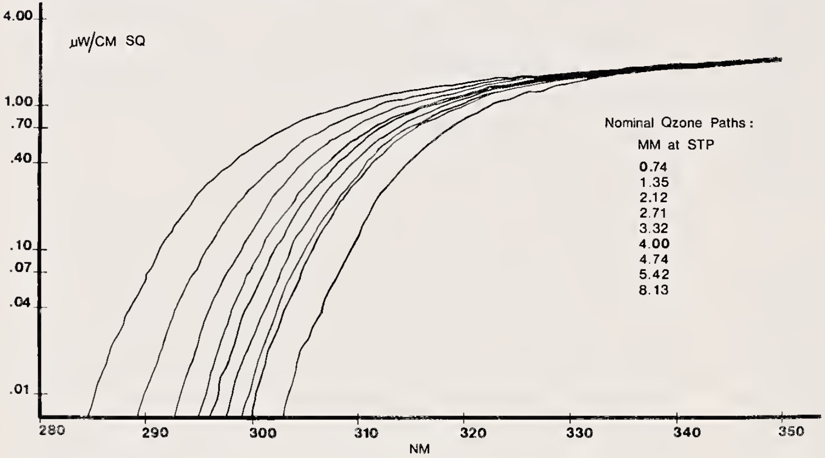
[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] Supported in part by Public Health Service contract N01-CP43271 with the Division of Cancer Cause and Prevention, National Cancer Institute, and grant ES00269 from the National Institute of Environmental Health Sciences.

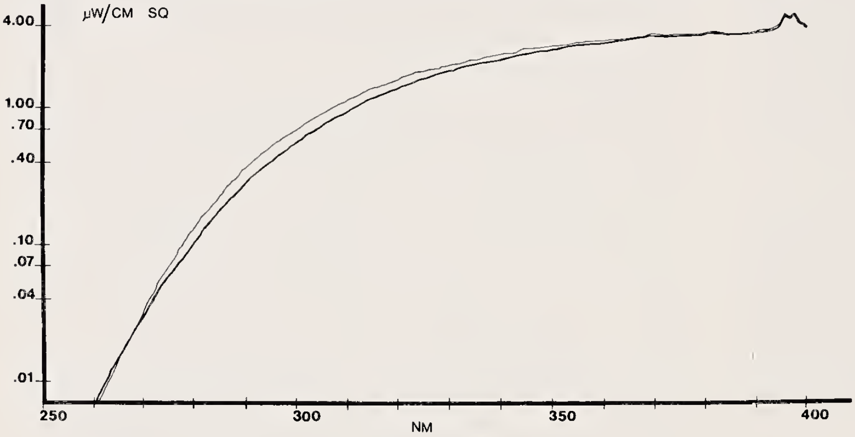
[3] Center for Photobiology, Skin and Cancer Hospital, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140.



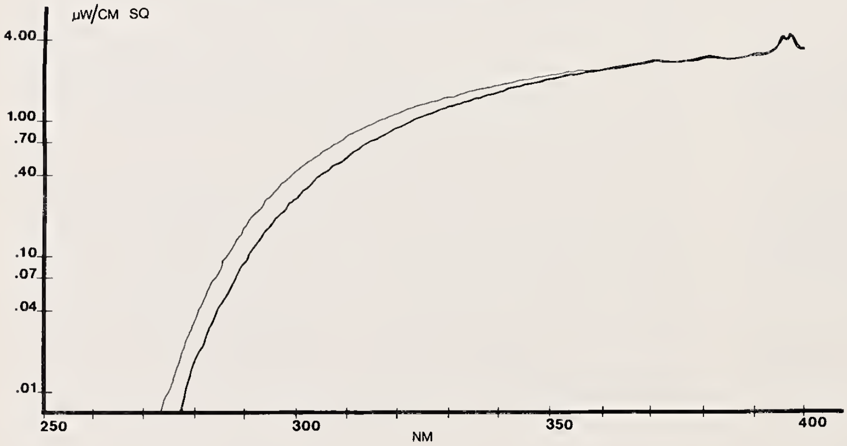
Text-figure 1.—Spectra of simulated solar UVR outside the earth's atmosphere *(upper curve)* and at ground level *(lower curve)*.



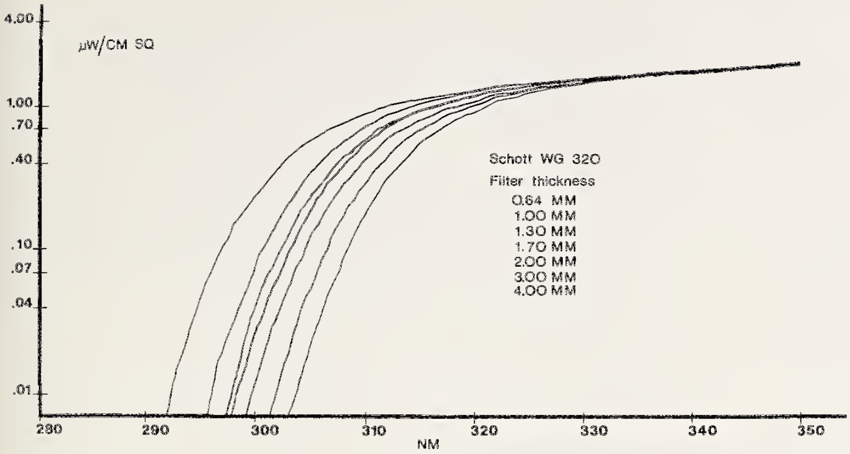
Text-figure 2.—Influence of nominal ozone path on UVR emission spectra (xenon lamp).



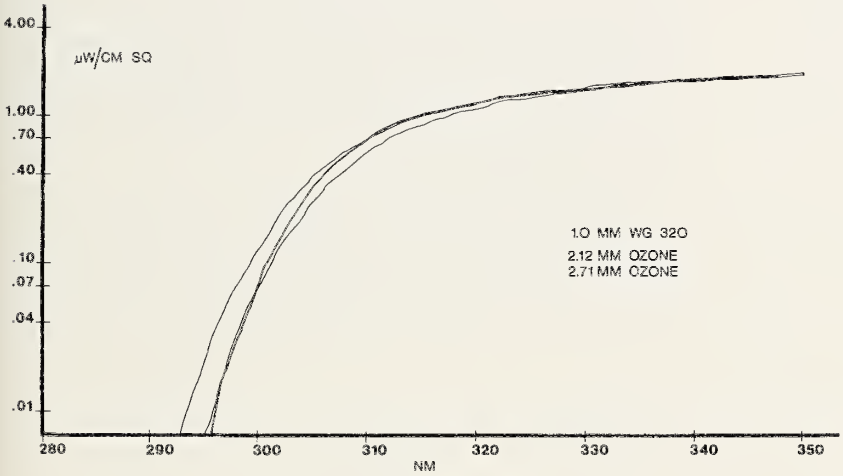
Text-figure 3.—Emission spectra from xenon lamp with new or used borosilicate filter.



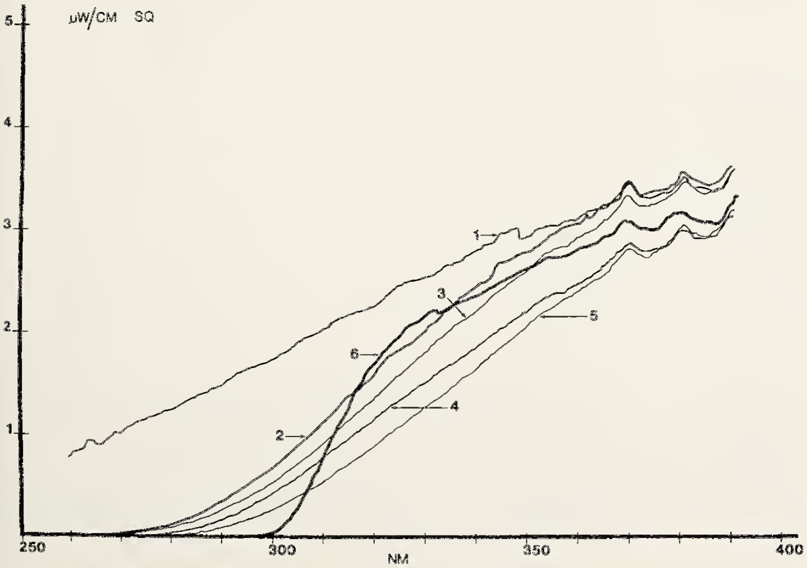
Text-figure 4.—Emission spectra from xenon lamp with new *(upper curve)* or used IR-absorbing filter.



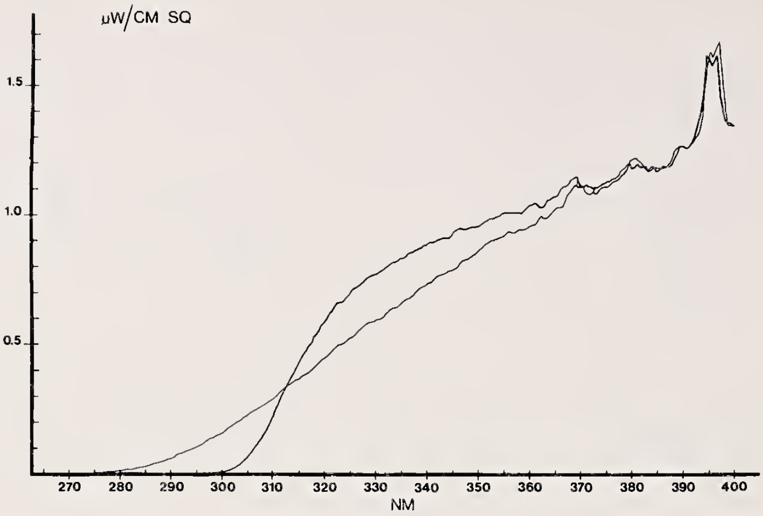
TEXT-FIGURE 5.—Influence of filter thickness on UVR emission spectra (xenon lamp).



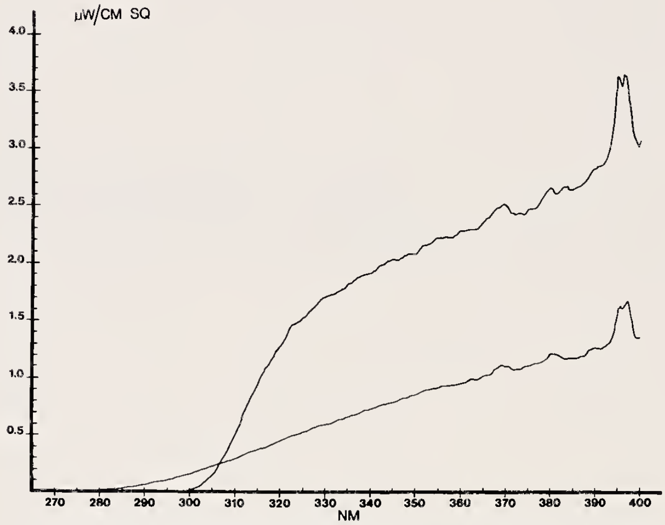
TEXT-FIGURE 6.—Emission spectra from xenon lamp, comparing the filtration effect of Schott glass (WG 320) with ozone.



TEXT-FIGURE 7.—Emission spectra from xenon lamp with borosilicate, IR-absorbing, or Schott (WG 320) glass filters.



TEXT-FIGURE 8.—Emission spectra from xenon lamp with WG 320 or IR-absorbing glass filters; *curves* matched for total integrated energy.

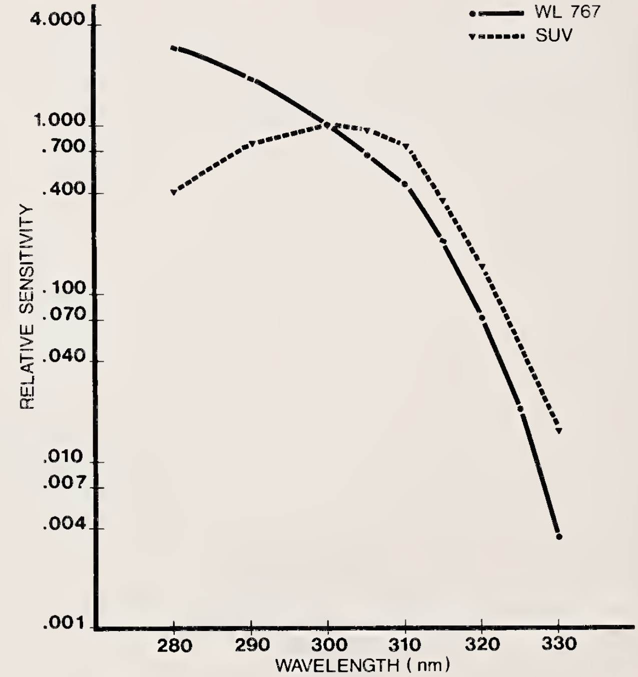


TEXT-FIGURE 9.—Emission spectra from xenon lamp with WG 320 or IR-absorbing glass filters; *curves* matched for sunburn effectiveness as predicted by R-B meter.

If we accept the WG 320 filter system as the most appropriate light *quality,* we are left with a decision on light *quantity.* We know from experiments that changing filters results in a modified sunburn effectiveness per unit time of exposure, despite the fact that *total energy* was only slightly different. Text-figure 8 gives a comparison of a WG 320 filter (upper curve at 320 nm) with an IR absorber. The total integrated energy under each curve is about 85 µW/cm², but the two spectra are not equally effective in producing biologic response.

For the moment, our decision on light quantity (as controlled by length of exposure) is based on two working hypotheses: 1) Carcinogenic and erythemogenic effectiveness are correlated; 2) erythemogenic effectiveness in mouse skin can be predicted by the SUV R-B sunburning meter (*7, 8*). More specifically, we set up current carcinogenesis experiments using the WG 320 filter to give the same R-B reading as other experiments with IR-absorbing filters. Text-figure 9 shows the same output spectra as in text-figure 8, now matched for sunburn effectiveness as predicted by the R-B meter. The curves obviously differ significantly in quality and in total integrated energy.

Two UVR measuring systems with which we have had considerable experience are designed to have UV spectral sensitivity similar to that for sunburn induction in human skin. When the two are normalized at 300 nm, the Westinghouse WL767 zirconium phototube and the current R-B



TEXT-FIGURE 10.—Spectral sensitivity of 2 UVR detectors (Westinghouse WL767 phototube and R-B meter), normalized at 300 nm.

meter react nearly the same to λ longer than 300 nm but diverge sharply below 300 nm (*see* text-fig. 10). Significantly, it is in this specific region (290–300 nm) that we encounter the most uncertainty about the shape of the human skin erythema action spectrum as well as the greatest likelihood of change in man's UV environment.

We have enough information to know that the WG 320 filtered system will produce tumors in mice and, therefore, that the system will be useful in examining possible interactive effects. What we cannot predict with certainty at this time is the absolute effectiveness of the system and, therefore, the time at which a given proportion of animals will be affected or the number of tumors that will appear. Preliminary evidence suggests that tumor development time will be longer than originally anticipated, which indicates that the R-B meter may either underestimate the relative carcinogenic effectiveness of λ less than 300 nm, or overestimate the contribution of longer wavelengths, or both. Since the WL767 instrument gives proportionately much greater weight to shorter wavelengths, we will also examine the effectiveness of doses equalized with respect to this response. A comparison of these and possibly other weighting functions, applied to various light sources, would provide some indication of the true shape of the carcinogenesis action spectrum.

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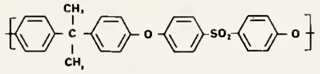
Description and Application of a Personal Ultraviolet Dosimeter: A Review of Preliminary Studies[1]

Anthony V. J. Challoner,[2] Margaret F. Corbett,[3] Anthony Davis,[4] Brian L. Diffey,[5] John F. Leach,[6] *and* Ian A. Magnus[7]

ABSTRACT—Preliminary work on a dosimeter suitable for measuring UVR exposure on individual persons is described. It is based on the use of polysulfone film which, on exposure, shows a change in absorbance that is determined spectrophotometrically. The results of studies of groups of persons with vastly different degrees of solar exposure, e.g., outdoor and indoor workers and geriatric patients, and psychiatric patients with photosensitivity from phenothiazine drugs are presented. We consider the dosimeter may also be suitable for epidemiologic studies in sunlight-induced skin cancer.—Natl Cancer Inst Monogr 50: 97–100, 1978.

Plastics, such as polysulfone and polyphenylene oxide, are known to darken when exposed to UVR, and the use of these materials in film form has been studied as possible monitors for measuring UVR. Polyphenylene oxide film shows sensitivity to UVR up to 400 nm, is about 10 times less sensitive than polysulfone, and has been used to monitor continuously solar UVR on a world-wide scale (*1*). Because polysulfone is more sensitive and only reactive to UVR below 340 nm, it seems more suitable for some biologic applications. Our remarks will be confined to use of this material as a dosimeter of UVR.

Two of the several requirements for an ideal UVR dosimeter or monitor (*2*) are that its response should be directly proportional to dose and independent of intensity. Also, in medical or physiologic applications, the wavelength response of the dosimeter should match the action spectrum of the biologic response to be studied, e.g., in skin erythema (sunburn) from terrestrial sunlight, the dosimeter should register UVR of 295- to 310-nm wavelengths only.



TEXT-FIGURE 1.—The structural unit of polysulfone. [Reproduced from *(1)* with permission of the publisher.]

PRELIMINARY DATA ON THE CHARACTERISTICS OF POLYSULFONE

Text-figure 1 shows the structure of polysulfone (Union Carbide P1700). Its thermal, thermal-oxidative, and high energy radiation stability have been demonstrated (*3–5*). However, the film readily degrades if exposed to UVR when its initial, i.e., preirradiation, broad absorption band in the near UV extends toward the visible spectrum. Changes in this band, in particular the increase in absorbance at 330 nm (ΔA_{330}), have been correlated with the UVR dose. If stored, exposed polysulfone film undergoes a slight dark reaction. For example, after 24 hours in the dark, ΔA_{330} may increase by about 4%, after 1 week by 5%. This dark reaction is eliminated by storage in a refrigerator.

Text-figure 2 shows the dose–response curve obtained for monochromatic radiation at 297 nm (half band width, 10 nm). A sixfold variation in intensity (56–337 μW/cm^2) did not affect the dose–response curve, i.e., within these limits, the reciprocity law was obeyed or dose = intensity × time. The dose–response curve of the film to UVR is nonlinear, probably because of the filtering effect of new absorption centers in the polymer from photolytic breakdown of the sulfone linkages (*1*).

The spectral sensitivity of the film was determined by measuring ΔA_{330} as a function of monochromatic UVR in the wavelength range of 254–335 nm. At each wavelength, measurements were made with increasing doses until ΔA_{330} reached approximately 0.3. When the dose–response curves for all wavelengths are normalized to eliminate the different wavelength sensitivities, the mean deviation of each curve from the 297-nm curve was less than 5%. Also, the response of a film to sunlight agrees with the 297-nm curve up to an absorbance of 0.2, but at higher absorbances, the agreement lessens.

The wavelength response of polysulfone film and the erythema action spectrum of human skin are compared in text-figure 3. Although the agreement is not ideal, it is possible to allow for this if the relative spectral distribution of the

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Abbreviations: UVR = ultraviolet radiation; W = watt(s); J = joule(s).

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[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

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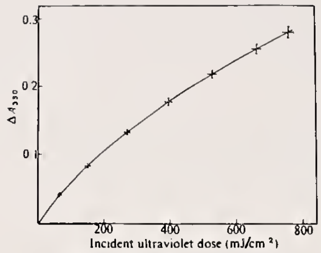


TEXT-FIGURE 2.—Calibration curve for relating change in absorbance of polysulfone film as a function of incident radiation at 297 nm. [Reproduced from *(1)* with permission of the publisher.]

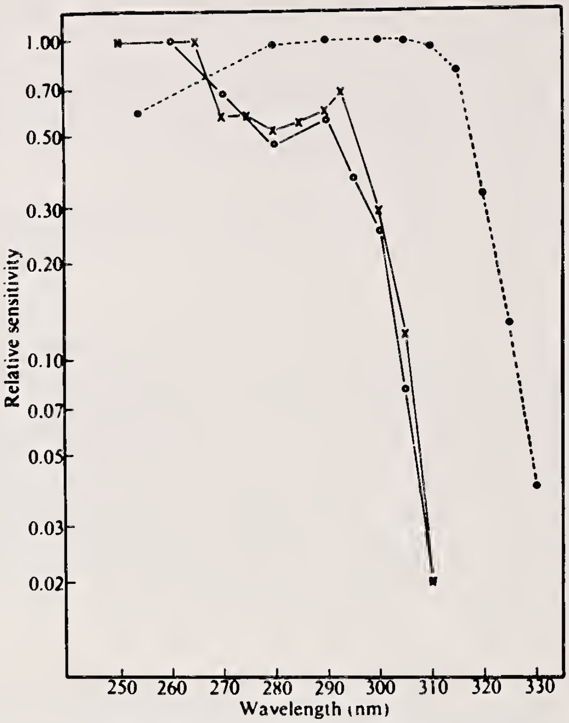
incident radiation is known. From a calibration curve, as shown in text-figure 2, it is possible to relate the change in absorbance of a film irradiated with heterogeneous UVR of known relative spectral distribution to an equivalent exposure of 297 nm radiation (*1*). The details of making these corrections for global sunlight of wavelengths 295–330 nm, based on the experimental measurements of Bener (*6*), are given by Diffey (*7*). Some of the uses of polysulfone film as a UVR dosimeter for humans will be outlined in the remainder of this paper.

PILOT TRIALS WITH POLYSULFONE FILM ON HUMAN SUBJECTS

Challoner et al. (*10*) compared the UVR received by laboratory workers, gardeners, and geriatric inpatients. The geriatric patients were divided into those able to sit in the sun on a balcony and those restricted to the ward. The observations were made during a 2-week period in June and July 1975. The gardeners worked in a semirural area, and the laboratory personnel were at a hospital complex within Metropolitan London.

The results (table 1) showed that the laboratory workers received about 5% of the ambient weekly dose and half of this was during leisure activities on the weekend, whereas the gardeners at work received just over 10% of the ambient weekly dose. In fact, the laboratory workers during the weekend showed mean daily exposures comparable to that of the gardeners at work, i.e., about 10 mJ/cm². The geriatric patients received doses ranging from about 0.1% of the ambient for those who were restricted to the ward to 9% for those who had access to the ward sun balcony. This study of the geriatric patients is now being extended to include their calcium and vitamin D metabolism and the effect of adding UVR from fluorescent sunlamps to the ward.

A similar investigation on the use of polysulfone film on a larger group of normal subjects over a longer time is in progress in Bristol, United Kingdom (Leach JF, McLeod VE, Pingstone AR: Unpublished observations). About 30 office personnel are being monitored for 8 fortnightly periods throughout winter and summer solstices, autumn and spring equinoxes, and points halfway between these. A preliminary analysis of the results indicates that the mean per person effective erythemal dose at 297 nm for this group may range from about 3 mJ/cm²/week in midwinter to about 60 mJ/cm²/week in midsummer. In keeping with the data of Challoner et al. (*10*) for laboratory workers, almost 50% of the dose was received on the weekend. The amount each person received (personal dose), about 3% of that available in the ambient global solar UVR, did not vary much with the season (text-fig. 4).

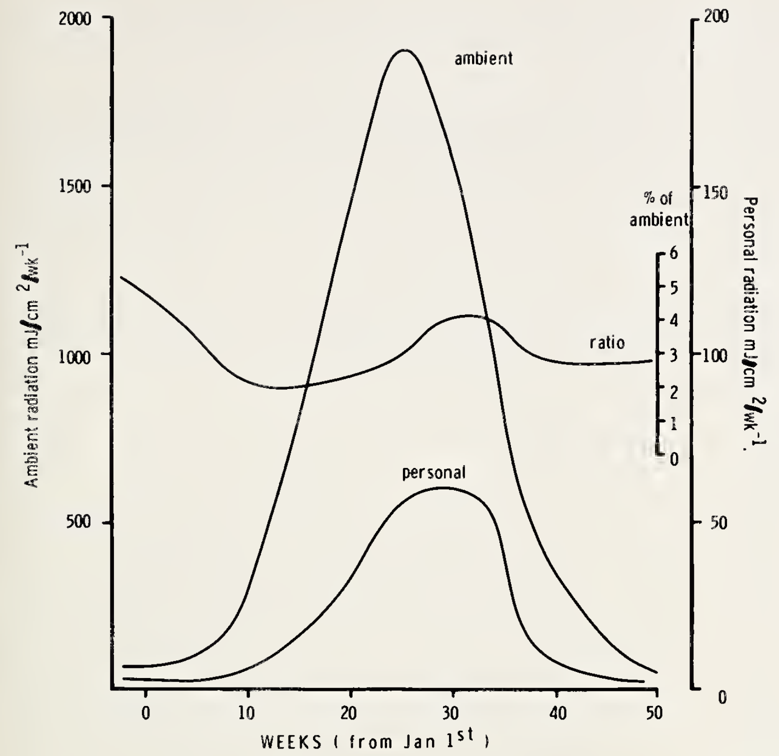


TEXT-FIGURE 3.—The UVR action spectra of polysulfone (40 μm thickness) for change in absorbance ● – – – ● and of human skin for erythema ○——○ and x——x *(8, 9)*. [Reproduced from *(1)* with permission of the publisher.]

In parallel with their field trials, Challoner et al. (*10*) compared the polysulfone response to that of an electronic UVR detecting system (*11*) by placing a static polysulfone film next to it. The detector head of this equipment and polysulfone film were positioned so that total UVR incidence in a horizontal plane was recorded. A linear relationship for UVR exposure was demonstrated by the 2 systems. Clearly in studies like the epidemiology of skin cancer, the polysulfone film as a personal badge dosimeter and a static electronic recorder in combination would be used with advantage.

PRELIMINARY STUDIES ON THE ANATOMICAL DISTRIBUTION OF SUNLIGHT

An important question is how measurements taken from a lapel badge dosimeter may be related to the dose distribution elsewhere on the body surface. This has been examined in preliminary studies (Diffey BL, Kerwin M, Davis A: Unpublished observations). Diffey et al. made 2-hour daily measurements, 1 hour each side of solar noon, on a tailor's display dummy. Ten polysulfone films were attached to vari-



TEXT-FIGURE 4.—Preliminary analysis of data from 1 yr of monitoring 30 office workers with a polysulfone UVR dosimeter. Mean points and SD omitted for clarity. *Abscissa scale:* wk from January 1, 1976. *Ordinate scales:* left refers to ambient curve; *right outer scale* to personal curve (i.e., mean of personal dosimeter values); *right inner scale* to ratio curve (i.e., ratio of mean personal to ambient curves). *Note* small and stable proportion (3–6%) of personal dose received compared with available ambient dose throughout the year.

TABLE 1.—*Preliminary field studies of UVR exposure with polysulfone dosimeters: Mean daily dose received in a work week by 3 groups of volunteers* [a]

Groups	Mean effective erythemal dose equivalent to 297 nm, mJ/cm² [b]
Polysulfone dosimeters	
Gardeners	21.8 ± 16.8
Laboratory workers	3.7 ± 1.4
Geriatric patients	
Exposed to sunshine on balcony	20.2 ± 16.7
Restricted to ward	0.23 ± 0.14
Static dosimeters	
Balcony of geriatric ward (partly shaded)	26.5 ± 10.3
Roof of nearby building	214.0 ± 82.0

[a] Data are from *(10)*.
[b] Values are mean ±SD.

ous parts of the surface of the dummy, which was rotated half a revolution per minute, to give some simulation of the random movements of the human body. Measurements were done in Canterbury, England (latitude, 51° N), during August and September 1976. At this location and during this period of the season, considerable variation in cloud cover occurs; the average zenith angle of the sun is about 40°.

The provisional findings may be summarized as follows. If the dose on the vertex, i.e., top of the head, is taken as 100%, that on the lapel badge and on most other approximately vertical surfaces of the body is about 60%. On approximately horizontal surfaces, such as the nose, the top of the shoulders (epaulet region), and the dorsum of the feet, where there will be some shading of direct sunlight by the rest of the body, the value is about 80% of the vertex. The cheeks and the back of the hands also receive approximately the same dose as other vertical surfaces, but the face dose can, of course, be considerably reduced, e.g., to about 30% or less, by a brimmed hat or long hair.

The above approximate values for anatomical distribution of UVR only apply to wavelengths approximating 297 nm and to the conditions at the time and place of that study. If the sun had been overhead, i.e., with a zenith angle about 0°, the badge dose for about 297 nm UVR on horizontal surfaces would be expected to be higher by a factor of about 3, whereas on approximately vertical surfaces, little or no change would occur. When the sun is overhead, as compared with it at a zenith angle of about 40°, the intensity of wavelengths around 297 nm increases from the sky by three times and direct from the sun by ten (*6*). Obviously, these considerations must be taken into account when results from such studies in different geographical locations are compared.

PILOT STUDIES OF MEDICAL APPLICATIONS OF THE DOSIMETER

We used the polysulfone film badge dosimeter to investigate abnormal skin photosensitivity in 32 psychiatric inpatients taking phenothiazines (Corbett MF, Davis A, Magnus IA: Unpublished observations). We assumed that the photosensitive action spectrum of these drugs is within 320–400 nm (*12*), which is a spectral range only partly within that of polysulfone (*1*). The medical and nursing staff recorded daily the hours that each patient under study spent outdoors in direct sunlight and his or her symptoms of photosensitivity. A simple system was used for deriving a photosensitive symptom score. Each patient wore the dosimeter badge, which was returned to us for spectrophotometric assay at the end of each week. On analysis, we found that badge dose and the hours recorded by the staff that each patient spent in sunlight had a strong positive correlation ($P < 0.001$). Thus the two methods for assessing patient exposure agree well. Secondly, the badge dose correlated positively with the symptom score for photosensitivity of the patients ($P < 0.001$). Thus we have, perhaps for the first time, objective evidence that the phenothiazines may indeed be photosensitizing; this may be laboring a clinically obvious point, but it does suggest that our method is sound. Lastly, symptom score was higher in patients on chlorpromazine compared with those on other phenothiazines ($P < 0.05$); this was not due to those patients receiving chlorpromazine being exposed to more sunlight, since the badge doses in the 2 treatment groups were not significantly different. Again, this is objective evidence for what was previously a clinical impression: Of the phenothiazine tranquilizers, chlorpromazine is the most likely one to photosensitize the skin. Yet another application of the polysulfone badge is to investigate individual instances of supposed photosensitivity. For example, we were consulted about the dermatosis which a certain cinema projectionist suffered. The question was: Did it arise from exposure to a high-pressure xenon arc light in the course of his work? The patient was asked to wear the polysulfone badge dosimeter while at work, and several of these were dispersed as static dosimeters in his projection room. After 2 weeks, all badges were examined and found to record negligible UVR exposure. All concerned (patient, dermatologist, employer, trade union) were then strongly assured that the patient's rash was not related to exposure to his projector lamp; we have heard no more about this patient. Therefore, the polysulfone film would seem to have a role in the investigations of safety aspects of UVR exposure. Other cases of alleged exposure to UVR, arising in the graphics printing industry, are under study.

COMMENT

We make no claims that this film badge dosimeter supersedes other methods of UVR monitoring, e.g., static electronic instruments, which give a different type of data. Rather, a personal film badge dosimeter gives information (if interpreted with care), which complements that from a static electronic monitor. Ideally, the two methods would be used in combination.

Polysulfone film could certainly be improved upon with respect to its action spectrum. For normal skin UVR erythema and, presumably, skin cancer, the film sensitivity extends a little too much toward the visible spectrum. For some drug photosensitivity studies, e.g., those of chlorpromazine, probably its long wavelength limit is too short. Nevertheless, it would seem to be a useful starting point, provided that the relative spectral distribution of the radiant source and the action spectrum of the biologic response under study are known.

Cheapness of material ($10.00/kg), ease of manufacture and of measurement (10 sec with a spectrophotometer), and the simplicity and smallness of the meter now offer the possibility that UVR dose measurements of biologic and medical interest, previously impractical, will now be feasible. Such measurements are possible on a large scale and, in field studies over large geographical areas, the film badges can be returned by post for processing to a central laboratory. Therefore, epidemiologic studies of skin cancer along these lines would seem possible.

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Workshop Summary: Ultraviolet Light Sources and Measuring Instruments, Calibrations, and Animal Models[1]

Claud S. Rupert [2]

This workshop was concerned with the physical apparatus and instrumentation of experimental UV carcinogenesis and environmental UV monitoring. A general introduction to the workshop by Dr. Rupert, a discussion by Dr. Forbes of light sources simulating the short-wavelength part of the normal sunlight spectrum, and a description of plastic "film-badge" UV dosimeters by Dr. Magnus are summarized in the preceding papers. The remaining presentations and comments are reviewed here.

Dr. Robert Freeman described the light source and monochromator system used in the study of the carcinogenesis action spectrum reported earlier in the meeting. This custom-built apparatus is constructed from commercially available monochromator units, for which replacement components were kept in stock to assure continuity of service throughout the experiments. It consists of three Bausch & Lomb grating monochromators, each with its own 150-W xenon arc light source, arranged in a common housing with the monochromator outputs converging at a single 1-cm exit port. These three outputs are slightly defocussed so as to intersect evenly over a distance from about 2.5 cm inside to 2.5 cm outside the port. (Skin, however, is usually irradiated exactly at the port position.) The changeable gratings on these units make the apparatus capable of operation over a range of wavelengths from the short-wavelength UV to the IR. It is also capable of delivering either one, two, or three separate wavelengths simultaneously to the same skin area with band widths from 2.5 to 20 nm. The output beam can be diverted to a photocell/amplifier with recorder for intensity monitoring before and after irradiation. Mouse irradiations are performed on the right ear after the animals are immobilized by inserting them head first into conical hardware cloth restrainers and pulling the ear through the wire mesh after they are in place. This container sits on a table mounted on a dental chair base, which provides for minute adjustments of height. For examining the erythemal action spectrum on human subjects, the table was replaced by a stool, allowing a series of closely spaced irradiated spots to be produced on neighboring areas of the same back or abdomen.

Dr. D. S. Berger spoke briefly about the use of a double monochromator (an instrument in which the dispersion and wavelength selection of the light are done twice in tandem) to minimize traces of extraneous wavelengths in the beam. He believes the quantitative accuracy on measurements of erythemal dose and erythemal action spectra amounts to about ±20% at best.

Dr. Berger went on to describe the design and performance of the Robertson-Berger sunburn UV meter. Since the bulk of this description has recently been published (*1*), it will not be summarized here, although it contributed a useful perspective to the workshop participants. A description of typical data received from the network of these instruments, stationed over the globe for the past several years, is also similar to recently published material to which the interested reader can refer [*see* (*9*) in Dr. Rupert's Introduction to the Workshop.) Stability of the meter response in the field is better than ±4% for 1.5 to 2 years, as checked by comparison with a previously calibrated meter sent to the field locations. The effect of the temperature on response is of opposite sign for wavelengths above and below 310 nm, and, consequently, the overall temperature coefficient differs with the spectral distribution of the light. Thus for sunlight, it also differs slightly with latitude and time of day. At noon in El Paso, it is less than −1% per degree Fahrenheit, but it is greater in the morning.

The discussion of dosimetric quantities and measurement units was to some degree a continuation of earlier discussions by the same participants (not complete without reference to previous debates). UV photobiology has lacked a comprehensive scheme of dosimetric quantities capable of dealing with all levels of phenomena from primary photochemical processes up to the overall effects in organisms. At the organism level, the dosimetric terminology has been generally satisfactory. The UV dose to the surface of an object (e.g., the skin) has been carefully and formally defined, and dermatologists have used this quantity consistently in their work. Those dealing with transparent suspensions of bacteria and viruses have informally used the same term in a different way, but they understood their fellow workers well. However, attempts to agree on a uniform terminology, applicable to all types of radiation, have highlighted these features of UV usage, as well as the difference in the meaning of "dose" as applied to ionizing and UV radiation. Although we have no great controversy about the facts, strong differences of opinion on what should be done about them do occur and tend to dominate discussions.

Rupert emphasized the distinction between the quantity that determines the photochemical effect of a light treatment at the site of the primary photoprocess (i.e., the fluence) and the quantity photobiologists formally define as "dose." Definitions of these two quantities and their relationship are outlined in his introductory paper to the workshop. Although the two quantities have the same physical dimensions (energy/area), they are distinct: The dose will, in general, have a different numerical value than the fluence at the same location. The only exception occurs when the light comes from a single direction and falls normally on the surface in question. For years, however, some authors have used the term "UV dose" to mean "UV fluence" also, whereas some editors, conscious of this practice, have recently tried to insist on the name "fluence" when an author was trying to describe the UV "dose" to the surface of skin. The two terms are not synonymous, nor is the one a modern replacement for the other.

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[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] Programs in Biology, The University of Texas at Dallas, P.O. Box 688, Richardson, Texas 75080.

The names for the quantities are particularly controversial. The meaning of the term "dose" as used in photobiology is wholly different from its meaning for ionizing radiation biology and complicates any common terminology. The name "fluence" (borrowed from ionizing radiation quantity, because no corresponding quantity had previously been defined for nonionizing radiation) is criticized by many on linguistic grounds, but no good substitute has yet been found.

Latarjet argued that the carefully constructed dosimetric terminology for photobiology should not be compromised in an effort to accomodate ionizing radiation terminology. To him the word "dose" is a term of broad meaning and usage and can represent different quantities in the various scientific fields. That it means different things for different radiations should not disturb anyone. The dose defined by photobiologists, rather than the fluence, is the relevant quantity for UV carcinogenesis, as shown by the fact that the anatomical areas of high skin tumor incidence are those which, on account of their average orientation, receive the highest long-term doses. These areas do not receive larger fluences. Latarjet doubts that fluence has much value in photobiology. Besides, he considers the name inappropriate, since it suggests something which flows, i.e., a flux, rather than an accumulation during a certain time of exposure.

Dr. J. C. van der Leun pointed out that Dr. Rupert's description of dose and fluence treated the latter as fundamental, and dose as a quantity of secondary character. However, if one starts with the photobiologic experiments, it is the dose which is directly encountered (and which can be directly measured by a thermopile), whereas the fluence arises in theoretical attempts to explain the mechanisms of the effects.

Dr. Rufus S. Day observed that it was time to stop haggling over the various possibilities and that the people involved should reach some conclusion as soon as possible, so that other workers going back to their laboratories could know how to report their work.

Dr. H. J. Kostkowski, of the United States Bureau of Standards, in response to some inconclusive dialogue, raised the question whether action-weighted dosimetric quantities (like that represented by equation 5 in Dr. Rupert's introductory paper) could be measured in units different from the unweighted parent quantity. This is done in experiments on vision, in which the action-weighted flux is measured in lumina rather than in watts. Unfortunately, this practice would lead to a new set of dosimetric units for each different action spectrum. One can avoid such an enormous proliferation of units by treating the action-weighted quantities as simply having the same physical dimensions as their parents and measuring them in the same physical units used for the parent quantities.

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SESSION IV

Repair Processes and Their Role in Carcinogenesis

Chairman: Kendric C. Smith

Co-Chairman: Betsy M. Sutherland

Introduction[1]

Kendric C. Smith[2]

One cannot help but be impressed with the multiplicity of the biochemical pathways of repair that a cell has at its disposal for maintaining the integrity of its DNA. Some pathways function in the absence of general macromolecular synthesis; others require normal growth conditions. One repair system is mediated by near-UV and visible light (photoreactivation), and another, having multiple pathways, removes damaged material from parental strands of DNA and replaces it with undamaged material (excision repair). One of these pathways makes short patches of repair synthesis, another makes long patches. Another multiple-pathway system bypasses the damage in the parental strands during DNA replication but leaves gaps in the daughter strands. These gaps are subsequently filled with material from the parental strands (postreplication repair).

There are repair systems that are constitutive, and at least one that is induced subsequent to the damage of DNA. Some repair pathways are error free, whereas others are error prone and produce mutations. Some genes are more mutable than others, as are different base pairs within a given gene. It is most important that we learn the factors that control this variability in site-specific mutagenesis.

Carcinogenesis also appears to be due to the mutagenic, error-prone repair of damaged DNA. There are diseases that predispose humans to an enhanced incidence of cancer, and skin cells from such patients have been shown to be deficient in one of the several pathways of DNA repair. The disease most relevant to this conference is xeroderma pigmentosum. Patients with this heritable disease are predisposed to sunlight-induced skin cancer at an early age. One group of patients with this disease is deficient in excision repair, whereas another group, the so-called xeroderma pigmentosum-variants, is deficient in postreplication repair. Thus the incidence of cancer can be enhanced by the genetic blockage of certain DNA repair systems.

Conversely, the incidence of UV radiation-induced skin cancer in animals can be reduced if the irradiated skin is treated with chemicals that have been shown to inhibit error-prone DNA repair in vitro. Since the modulation of repair systems, either genetically or chemically, can affect the incidence of mutagenesis and carcinogenesis, it offers the possibility of cancer prevention by the enhancement of error-free repair systems and/or inhibition of error-prone systems by drugs and/or genetic engineering.

Since the molecular basis of carcinogenesis appears to be the error-prone repair of damaged DNA, it follows that a complete understanding of the molecular mechanisms of DNA repair and mutagenesis will be required before a complete understanding of carcinogenesis can be achieved. Thus the study of DNA repair, which formerly was only a hobby of a few radiation biologists, is now, or should be, the most important thrust of cancer biology.

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[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

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Multiple Pathways of DNA Repair and Their Possible Roles in Mutagenesis[1, 2]

Kendric C. Smith[3]

ABSTRACT—In studies on bacteria, the *excision repair* of UVR-induced DNA base damage has been divided into two major pathways on the basis of physiologic requirements and genetic control. The major pathway requires a functional *polA*$^+$ gene, does not need complete growth medium, is largely error free, and produces short patches during repair. The second pathway requires complete growth medium and functional *recA*$^+$, *recB*$^+$, *recC*$^+$, *lexA*$^+$, *uvrD*$^+$, and *polC*$^+$ genes, is mutagenic, and produces long patches during repair. A second type of excision repair exists, in which the modified base is removed by a DNA glycosylase, and the chain is nicked by an apurinic (apyrimidinic) acid endonuclease. Subsequent events are presumed similar to the above excision repair process. The *postreplication repair* system has been divided into at least four distinct pathways, three of which depend on functional *recB*$^+$, *lexA*$^+$, and *uvrD*$^+$ genes, and are error free. A fourth pathway depends on the above gene products but is blocked by postirradiation treatment with chloramphenicol, and may be the UV-inducible, error-prone, mutagenic pathway of repair ("SOS repair"). A possible fifth pathway is dependent on a functional *recF*$^+$ gene and is independent of the *recB*$^+$-dependent pathway. *Mutagenesis* is the result of error-prone DNA repair, and evidence is growing that carcinogenesis is also the result of error-prone repair. Therefore, a complete understanding of DNA repair is crucial to a complete understanding of the molecular basis of carcinogenesis.—Natl Cancer Inst Monogr 50: 107–114, 1978.

The discovery that cells can recover from radiation-induced damage has provided a major stimulus to the field of radiation biology. The more recent observations that DNA repair functions are necessary for normal metabolic processes, i.e., certain repair deficiencies are lethal in combination with the *polA* mutation [*see* references cited in (*1*)], and that error-prone repair is the molecular basis of mutagenesis (*2*) and of carcinogenesis (*3, 4*) mean that DNA repair can no longer be considered the private property of radiation biology. The complete understanding of DNA repair is now required for a proper understanding of normal cellular metabolism, mutagenesis, carcinogenesis, and even of aging (*5*).

Our greatest insight into the complexity of DNA repair, i.e., the diverse systems of repair and the multiply biochemical pathways within these systems, has come from bacterial studies because of the availability of 1) numerous radiation-sensitive mutants (*6, 7*), 2) the techniques of molecular genetics that permit the construction of multiply repair-deficient mutants, and 3) the techniques of molecular biology that permit one to follow the repair of DNA at the molecular level. With the recent isolation of repair-deficient human cell lines, such as xeroderma pigmentosum (*8*) and ataxia telangiectasia (*9*), similar genetic studies are now becoming possible with mammalian cells.

Three modes of DNA repair have been documented.

1) The damaged part of the molecule is restored to its functional state in situ. This may result from the spontaneous "decay" of the damage to an innocuous form, e.g., dehydration of pyrimidine photohydrates or the recombination of radicals to yield a restored molecule; or it may be accomplished by some enzymatic mechanism, e.g., photoreactivation. *Photoreactivation* is the enzymatic splitting of cyclobutane-type pyrimidine dimers in situ mediated by exposure to visible light.

2) The damaged section of a DNA strand is excised and replaced with undamaged nucleotides to restore the normal function of the DNA. This *excision repair* system constituted the first discovery of a mechanism of "dark repair" (to distinguish it from photoreactivation mediated by visible light). This excision repair system repairs various radiation-induced and chemically-induced lesions in DNA, but it was originally observed as a mechanism for the repair of UVR-induced cyclobutane-type pyrimidine dimers (*10*).

3) The damage itself is not repaired but instead is bypassed during replication, leaving gaps in the daughter strand DNA; the missing genetic information is subsequently supplied by redundant information within the cell. This type of repair is called "*postreplication repair,*" since it occurs in the DNA synthesized subsequent to UV irradiation (*10*).

Although the procedures for delineating the multiple pathways of DNA repair in bacteria are straightforward in principle, they are often not so simple in practice. After one isolates a group of radiation-sensitive mutants and maps the genes conferring this sensitivity, the next step is to determine at the molecular level what type of repair event is blocked in these mutant strains. Then strains are constructed to contain two or more mutant genes that affect radiation sensitivity. Multiple pathways are implicated when, e.g., a double mutant is more sensitive to killing by radiation than are either of the singly mutant parent strains, and this reduced survival capacity is correlated with deficiencies in repair capacity, which is measured at the molecular level. When the most important pathways of the major repair systems are known (current level of knowledge), the genetic control of all the steps involved in each of the pathways must be determined (will require many more years of research) and, ultimately, the gene products must be isolated and their properties investigated in repair systems in vitro.

Abbreviations: UVR = ultraviolet radiation; LHR = liquid holding recovery.

[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] Supported by Public Health Service grants CA02896 and CA06437 and research project grant CA10372 from the National Cancer Institute.

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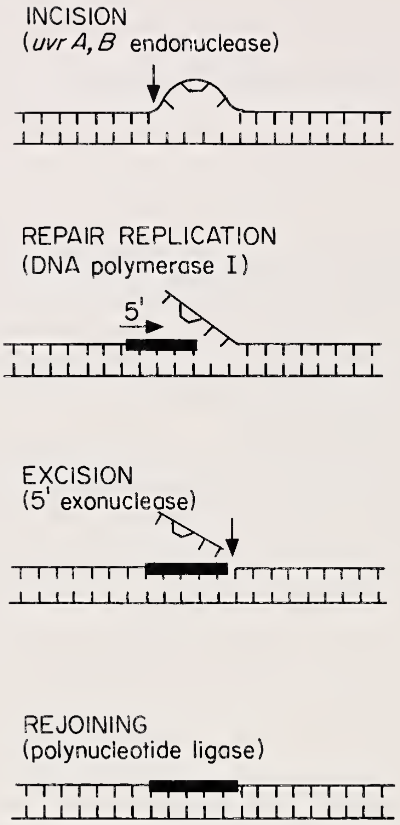
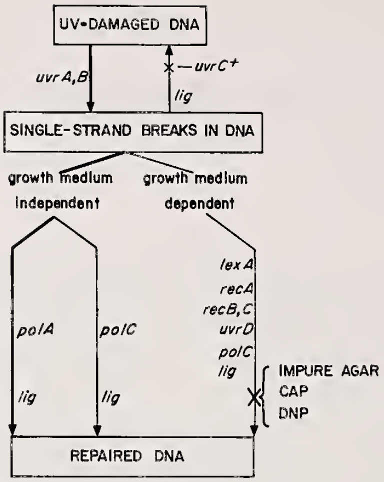


TEXT-FIGURE 1.—A general model for excision repair. The repair patch is shown as a *heavy line. Vertical arrows* signify locations of nuclease cuts in the damaged DNA strand, and the *horizontal arrow* indicates the direction of repair replication, beginning at the 3'OH end of the DNA strand.



TEXT-FIGURE 2.—Genetic and physiologic control of the different pathways of excision repair. [Modified from (*18*).]

Even at the current level of knowledge, we can ask the question: Which of the multiple pathways of repair are error free and which are error prone, i.e., lead to mutagenesis? Again, in principle, this question should be easy to answer, but, in practice, this is not so.

This report summarizes current knowledge on the multiple pathways of the various types of DNA repair and indicates, when possible, which pathways are error free and which are error prone.

EXCISION REPAIR

polA^{+}-Dependent Pathway

From the early work of Setlow (*11*), Riklis (*12*), Howard-Flanders (*13*), Hanawalt (*14*), and others came a prediction of the probable steps involved in excision repair, shown schematically in text-figure 1.

The first steps in the excision repair system are the recognition of damage and the introduction of a break in the DNA chain near the lesion (incision step). Resynthesis is then initiated by the action of DNA polymerase (repair replication), by the use of the opposite strand of DNA as the template. The lesion is cut out to complete the excision process and, finally, when the excised region is filled with undamaged nucleotides, the single-strand interruption is closed enzymatically by polynucleotide ligase, yielding repaired DNA. Although this has proved to be the general mechanism for the major pathway of excision repair, it is not the only pathway.

The enzyme responsible for making an incision break in the DNA at the site of the damage is coded for by the *uvrA* and *uvrB* genes (*15*). In a strain carrying a mutation at *uvrC*, most of the incisions produced by the *uvrA,B* endonuclease are apparently resealed by polynucleotide ligase (*16*) and thus thwart the excision repair process. Polynucleotide ligase has also been shown to reseal *uvrA,B* endonuclease incisions in vitro (*17*).

After the initial incision event, excision repair can be divided into two pathways on the basis of physiologic requirement and genetic control (text-fig. 2) (*18*). In one pathway, repair can occur in buffer and requires DNA polymerase I (*polA*$^{+}$); it probably proceeds according to the scheme shown in text-figure 1. In the absence of DNA polymerase I (i.e., in a *polA* strain), DNA polymerase III (*polC*$^{+}$), and/or DNA polymerase II (*polB*$^{+}$) can partially substitute (about 25% as efficiently) (*18*). In cells of a *polA polC* strain that had been subjected to toluene, DNA polymerase II functioned in repair replication (*19*). However, a single mutation at *polB* does not seem to affect cellular sensitivity to either UV (*20, 21*) or X-irradiation (*22*), which suggests that DNA polymerase I and III do most of the repair synthesis in vivo. Polynucleotide ligase is required to reseal the repaired strands of DNA in vivo (*23*).

Since a *polA uvrA* strain is only slightly more sensitive to UVR than is a *uvrA* strain (*24*), most excision repair is probably accomplished via the *polA*$^{+}$-dependent pathway. This pathway apparently produces short patches of repair replication (less than 30 nucleotides long) (*25, 26*). The enzymology of excision repair was reviewed recently (*27, 28*).

The possibility of a new excision repair process that works on a photoproduct other than cyclobutane-type pyrimidine dimers has been reported (*29*). DNA single-strand breaks are observed in *uvrA* and *uvrB* strains of *Escherichia coli* after high fluences of UVR, and their yield is not affected by photoreactivation. These breaks are repaired by a process that requires DNA polymerase I and polynucleotide ligase but does not require the *recA*$^+$, *recB*$^+$, *recF*$^+$, *lexA*$^+$, or *uvrD*$^+$ gene products (*29*).

recA$^+$-Dependent Pathway

A second pathway of excision repair requires complete growth medium, and functional *recA, recB, lexA* (*18*), *uvrD* (Youngs DA, Van der Schueren E, Smith KC: Unpublished observations), *lig* (*23*), and *polC* (*30*) genes (text-fig. 2). This pathway produces long patches of repair replication ($\sim$ 1,500 nucleotides long), since long patch repair was not observed in a *recA recB* strain (*26*). However, such repair was greatly enhanced in a *polA* strain (*26*), which is blocked in the major pathway of excision repair.[4] From considerations discussed in the previous section, the *recA*$^+$-dependent pathway probably handles many fewer lesions than does the *polA*$^+$-dependent one.

The growth medium-dependent pathway of excision repair is irreversibly inhibited by an 80- to 90-minute postirradiation treatment with 50 μg chloramphenicol/ml (*18*) or 10^{-2} M dinitrophenol that selectively blocks a *lexA*$^+$-dependent repair function (*31*). The growth medium-dependent pathway is also blocked by impurities in all brands of regular agar that have been tested but is not inhibited by washed Noble agar (*32*).

The model for excision repair shown in text-figure 1 should be classified as *prereplication* excision repair, since normal DNA replication is not required. Some investigators have suggested that the *uvrA* and *uvrB* genes may influence postreplication repair [(*33, 34*); Rothman RH, Clark AJ: Unpublished observations], and models for postreplication excision repair have been advanced [(*34*); Rothman RH, Clark AJ: Unpublished observations]. If there is an excision repair system that does depend on DNA replication, then the growth medium-dependent pathway shown in text-figure 2 is the most likely candidate for such a system.

Liquid Holding Recovery

When UV-irradiated cells of certain strains of *E. coli* are held in buffer for several hours before plating on nutrient medium, they show a much higher survival than if plated immediately. This phenomenon, now called "liquid holding recovery," was first described for bacteria with the use of *E. coli* B (*35*) but is also observed in *recA* strains of *E. coli* K-12 (*36*). Because mutations at *uvrA, uvrB,* or *uvrC* block LHR (*37*), the molecular basis of LHR is excision repair. Since LHR occurs in buffer, it seems logical that the pathway of excision repair involved in LHR is the *polA*$^+$-dependent pathway. Since this pathway proceeds in buffer anyway, what is the basis of the enhanced survival? After preparing various double mutants in combination with *recA,* I have observed (unpublished) that *uvrD* blocks LHR, *recF* has little effect on LHR, and *lexA* and *recB* mutations in combination with *recA* greatly enhance LHR over that observed in *recA* alone. One interpretation of these results is that some of the excision gaps processed by the growth medium-dependent pathway may be modified, especially in *recA lexA* and *recA recB* strains, such that they become a substrate for the *polA*$^+$-dependent pathway of excision repair. In growth medium, these excision gaps would be converted to lethal lesions but in buffer, they may be shunted to the *polA*$^+$-dependent pathway, be properly repaired, and result in enhanced viability. Other interpretations of these data are possible, and we are continuing our investigation of the genetic control and molecular basis of LHR.

Thus besides the existence of multiple, independent pathways of repair, under certain conditions, shunts may be opened between two normally independent pathways. Such a shunting mechanism further attests to the ingenuity of cells in DNA repair. [Evidence has been presented for a shunt between the *recB* and *recF* pathways of genetic recombination (*38*).]

Repair of X-ray-Induced DNA Single-Strand Breaks

The first step in the excision repair of UVR-induced DNA base damage is the enzymatic production of a single-strand break in DNA. X-irradiation, besides producing base damage, also produces breaks in DNA strands by radiation chemical mechanisms. Town et al. (*39*) have shown that X-ray-induced DNA single-strand breaks are repaired by the same general pathways described in text-figure 2. Thus there is a growth medium-independent pathway (type II repair) that requires DNA polymerase I (*polA*$^+$). In a *polA* strain, DNA polymerase III (i.e., *polC*$^+$) can substitute in part (*40*). Type II repair is rapid ($T_{\frac{1}{2}}$: $\sim$ 10 min at 0° C, $\sim$ 1 min at 37° C) and repairs approximately 90% of the X-ray-induced DNA single-strand breaks.

Although the growth medium-dependent system (type III repair) is slow (requires $\sim$ 50 min at 37° C) and repairs only a few breaks, it is clearly important to the survival of X-irradiated cells (*39*). This repair pathway is irreversibly inhibited by a 90-minute postirradiation treatment with 40 μg chloramphenicol/ml (*41*) or 3×10^{-3} M dinitrophenol (*42*), which is a lower concentration of dinitrophenol than is required to inhibit this pathway maximally after UV irradiation (*31*). Another difference in this pathway for repair after UV or X-irradiation is that, in contrast to UV irradiation, this pathway is *not* inhibited by impurities in the regular agar used in growth plates (*32*). It seems reasonable that some differences should occur in the steps in this pathway for the repair of breaks produced enzymatically after UV irradiation and the breaks produced by radiation chemistry during X-irradiation.

Excision Repair of X-ray-Induced Base Damage

Few data are available on the excision repair of X-ray-induced base damage, primarily because no easy assay for this type of damage is available. Hariharan and Cerutti (*43*) have developed an indirect assay that measures one type of thymine base damage produced by ionizing radiation.

Endonucleolytic activity that recognizes X-ray-induced

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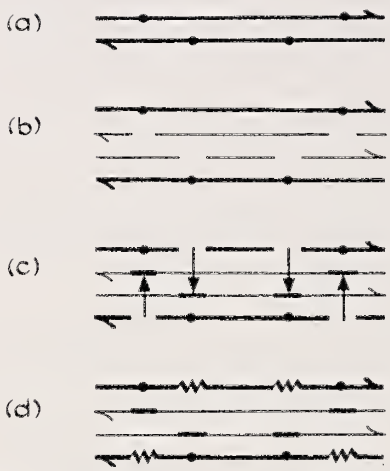
[4] Recent data (Carlson KM, Smith KC: Unpublished observations) indicate that in *polA*$^+$ strains, long patch excision repair requires DNA polymerase II (i.e., *polB*$^+$).



TEXT-FIGURE 3.—A general model for the postreplication repair of UV radiation-damaged DNA. a) *Dots* indicate photochemical lesions produced in the two strands of DNA. b) DNA synthesis proceeds past the lesions in the parental strands leaving gaps in the daughter strands. c) Filling of the gaps in the daughter strands with material from the parental strands by a recombinational process (depends on functional $recA^+$ gene). d) Repair of the gaps in the parental strands by repair replication.

nucleotide damage in double-stranded DNA was detected in extracts of *Micrococcus luteus,* HeLa, *Bacillus subtilis,* and *E. coli* cells [(*44*) and references therein].

The elucidation of the mechanisms by which cells repair X-ray-induced DNA base damage is one of the major challenges for the coming years.

Base Excision Repair

The excision repair system described above may be classified as a nucleotide excision process, since the whole damaged region is excised. A new type of excision repair process, base excision repair, has been formulated recently [(*28*) and references therein]. In this process, an altered base is recognized and cut from the sugar-phosphate chain by a DNA glycosylase. An enzyme specific for uracil residues in DNA and other enzymes specific for alkylated bases have been isolated.

The action of a DNA glycosylase leaves an apurinic (apyrimidinic) site that is then susceptible to attack by a specific endonuclease. Presumably after this incision event, subsequent excision and resynthesis occurs in a manner similar to that described above for the more usual excision repair system (*28*). Much more data are needed to clarify this new mode of excision repair.

POSTREPLICATION REPAIR

The DNA that is synthesized shortly after UV irradiation in cells of *E. coli* K-12 has discontinuities when assayed in alkaline sucrose gradients. In excision-deficient cells, the mean length of newly synthesized daughter-strand DNA approximates the average distance between pyrimidine dimers in the parental strands. With further incubation of the cells, however, these discontinuities disappear, and the DNA approximates the molecular size of that from unirradiated control cells (*45*). These results suggested a postreplicational mode of repair, shown schematically in text-figure 3.

DNA replication proceeds past the lesions in the parental strands and leaves gaps in the daughter strands that are filled with material from the parental strands by a recombinational process. The gaps formed in the parental strands are then filled by repair replication.

The size of the daughter-strand gaps appears to be about 1,000 nucleotides long (*46*). Evidence has been obtained that parental and daughter-strand DNA become covalently attached during postreplication repair (*47*), as would be predicted by the model in text-figure 3. Perhaps the best evidence that this is true comes from the observation that about half the sites in UV-irradiated DNA that are sensitive to the action of the T4 UV endonuclease (i.e., pyrimidine dimers) are transferred from the parental strands to the daughter strands during postreplication repair (*48*). This finding implies that it may take several rounds of replication and postreplication repair before the DNA lesions are "diluted out" and a viable strand of DNA is obtained. This idea is consistent with the observation that about 5 hours are required to eliminate the effect of pyrimidine dimers on viability in an excision repair-deficient strain after a UV fluence of 6.3 J/m^2 (∾ 65% survival) (*49*).

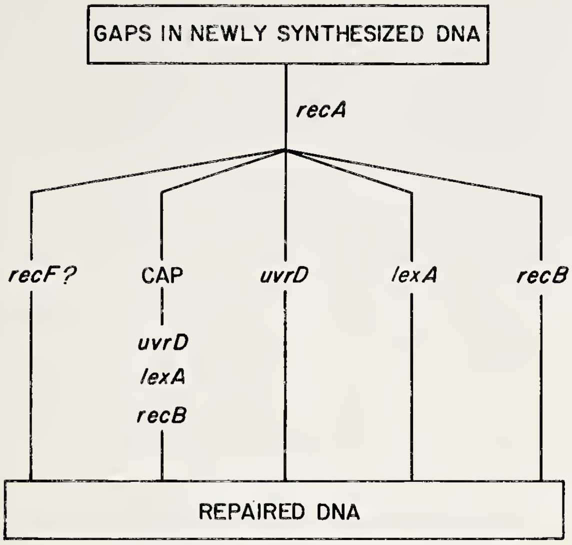
On the basis of genetic and molecular biologic studies (*50*), postreplication repair has been divided into several independent pathways (text-fig. 4). The filling of the gaps in newly synthesized daughter strands of DNA is inhibited in *recA* mutants (*51, 52*). The *recB, uvrD* (*50*), and *lexA* (*50, 53*) strains are only partially deficient and additive (*50*) in their ability to repair the gaps. Chloramphenicol partially blocks the filling of gaps (*50, 53*), but not if the cells are mutant at either *lexA* (*50, 53*), *uvrD,* or *recB* (*50*). Thus in addition to their independent functions, another pathway may be present that needs the cooperation of all three of these gene products and also requires the synthesis of protein after UV irradiation (*50*).

Since a *uvrB lexA uvrD recB* strain is not quite as UV sensitive as is a *uvrB recA* strain, at least one other pathway of postreplication repair must exist (*50*). The *recF* gene may code for such a pathway, since the *recF* mutation has been shown to act independently of *recB* in its effect on postreplication repair (*54*). No one has determined whether *recF* is independent of *lexA* and *uvrD.*

Efficient postreplication repair can occur in either *polA* or *polC*ts strains (*55, 56*), but it is blocked in a *polA polC*ts strain at the restrictive temperature (*55–57*). This result does not tell us which of the two DNA polymerases (i.e., I or III) is the preferred enzyme in wild-type cells. Polynucleotide ligase is required for the completion of postreplication repair (*23*).

MUTAGENESIS

The *uvrA* and *uvrB* strains appear to be more mutable than wild-type strains. This led to the suggestion that postreplication repair, apparently the only type of dark repair remaining in a *uvrA,B* strain, produces mutations due to inaccurate repair (*58, 59*). In support of this concept are the observations that *recA* (*60, 61*) and *lexA* (*62*) strains, which are



TEXT-FIGURE 4.—The genetic control of the different pathways of postreplication repair. [Modified from *(50)*.]

deficient in postreplication repair, cannot be mutated by UV radiation. However, the *recA* and *lexA* strains are also deficient in the growth medium-dependent pathway of excision repair (text-fig. 2), which has been implicated in UVR-induced mutagenesis (*63–65*). Taken together, these data suggest that the *polA*$^+$-dependent pathway of excision repair must be largely error free.

Thus three major pathways of repair may affect mutagenesis: 1) the *polA*$^+$-dependent pathway of excision repair appears to be error free (i.e., produces no mutations), 2) the *recA*$^+$ *lexA*$^+$-dependent pathway of excision repair produces mutations, and 3) postreplication repair must be partially error prone (i.e., mutagenic). What data support these concepts?

It would seem easy enough to test these concepts: One simply needs to compare the UVR-induced mutability of the various repair-deficient strains with that of the wild-type strain, using "classical" techniques. Unfortunately, the interpretation of such results is often not easy (*66*).

The fact that no UVR-induced mutations are produced in *recA* or *lexA* strains (*60–62*), strains whose *polA*$^+$-dependent pathway of excision repair is fully functional (*18*), suggests that the *polA*$^+$-dependent pathway is error free and leads to the prediction that mutagenesis should be greatly enhanced in a *polA* strain. The mutagenesis data are confusing, however, and do not entirely support this hypothesis.

The mutability (i.e., mutants per survivor as a function of UV fluence) of *polA* strains was reported to be greater than wild-type strains up to a fluence of 10 J/m^2 (∾ 50% survival for *polA*), but the same as wild-type strains at higher fluences (*67*). However, if these data are compared at equal survival rather than at equal fluence, then a *polA* strain shows *less* mutability than a wild-type strain (*66, 68*). If the data are plotted yet another way, e.g., as a function of UV fluence but on linear coordinates, they indicate that a *polA* strain is only slightly more mutable at fluences up to 10 J/m^2 and thereafter is markedly *less* mutable than the wild-type strain (*69*). Thus *polA* strains seem to be more mutable than wild-type strains at fluences at which the *polA* strain shows greater than approximately 50% survival, but at higher fluences is interpreted to be either the same or less mutable, depending on how the data are plotted.

Whereas the results at the lower fluences are consistent with the idea that the *polA*$^+$-dependent pathway of excision repair is error free, the data at the higher fluences are not. These data call to our attention that different conclusions can be reached concerning the mutability of repair-deficient strains depending on how the data are plotted (*66*) and on the range of fluences of UVR used.

What about the different pathways of postreplication repair? Which are error prone and which are error free? One pathway of postreplication repair was blocked by postirradiation treatment with chloramphenicol, which suggested that protein synthesis is required after UV irradiation before this pathway can function (*50, 53*). This pathway has also been shown to require functional *uvrD, recB* (*50*), and *lexA* (*50, 53*) genes (text-fig. 4). UVR mutagenesis is blocked by postirradiation treatment with chloramphenicol (*53*), which suggests that the *uvrD, lexA,* and *recB* gene products are involved in mutagenic repair. The fact that UVR-induced mutations are not produced in *lexA* strains (*62*) seems to support the idea that the chloramphenicol-inhabitable pathway is the mutagenic pathway.[5]

When compared at equal fluence, the *uvrD* and *uvrA uvrD* strains show the same mutation frequency as their respective *uvrD*$^+$ parent strains, which suggests that the *uvrD* gene product is not involved in mutagenic repair. However, if the same data are plotted as a function of equal survival, then the *uvrD* strains show less mutability and thus implicate the *uvrD* gene product in error-prone repair (*66*). Which interpretation is correct? The question has only been partially resolved (*66*). A similar dilemma exists for the *recF* strain (Rothman RH, Clark AJ: Unpublished observations).

The data for the *recB* and *recC* strains are also difficult to interpret. Reversions to phototrophy seem to be slightly reduced in *recB* or *recC* strains (*71*). Mutations in *recC* that were scored selectively (e.g., Mal$^+$) were reduced but mutations that were scored nonselectively (i.e., Lac$^-$) were not reduced. Hill and Nestmann (*72*) postulated that lethal sectoring was responsible for the reduced yields of mutations that were scored selectively. Thus any conclusion as to whether the *recB* and *recC* gene product (Exo V) is involved in mutagenesis depends partly on how the experiments are performed.

Regrettably, classical studies on the relative mutagenicity of repair-deficient strains compared with wild-type strains frequently do not permit an easy answer to the question of which repair pathways are error prone and which are error free; resolution of this dilemma depends on the success of future research.[6]

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[5] The current concept that error-prone, mutagenic, postreplication repair is inducible (*2, 70*) is consistent with the data that chloramphenicol inhibits mutagenesis (*53*) and one pathway of postreplication repair (*50, 53*).

[6] Recent data (Sargentini NJ, Smith KC: Unpublished observations) indicate that *uvrB* strains additionally deficient in *uvrD*$^+$ or *recB*$^+$ functions show a reduced UVR-induced mutability; however, strains that are deficient in both the *uvrD*$^+$ and *recB*$^+$ functions are nonmutable by UVR. Thus there appear to be two distinct mutagenic pathways of postreplication repair.

CONCLUSIONS

One cannot help but be impressed with the multitude and sophistication of the repair pathways possessed by cells. One system (excision repair) can repair damage in parental strands of DNA under nongrowth conditions, whereas another repairs gaps in daughter strands that can only be produced in complete growth medium (postreplication repair). A significant percentage of the energy of a cell is spent in synthesizing enzymes to repair and maintain the integrity of the genetic code in its DNA. However, some evidence does suggest that these repair systems were not developed just to repair radiation or chemical damage to DNA but also appear to have a necessary function in the everyday life of a cell.

According to current concepts, there are three types of repair events: 1) Error-free repair (mainly excision repair) produces no mutations and no lethality; 2) error-prone repair (mainly an inducible pathway of postreplication repair) produces mutations that can either be lethal or nonlethal; and 3) incomplete repair (i.e., failure to reestablish the continuity of the strands of DNA) is lethal and nonmutagenic in bacteria. Repair-deficient strains would be expected to perform these repair events in relative proportions different from those performed by the wild-type strain. This being so, it is difficult to interpret classical mutation frequency data with the goal of determining which of the many pathways of excision and postreplication repair are error free and which are error prone.

Since radiation-induced mutagenesis results from the error-prone repair of damaged DNA, and nearly all ultimate carcinogens are mutagens, it follows that carcinogenesis may also be due to errors in the repair of damaged DNA. Thus a complete understanding of DNA repair is essential to a complete understanding of the molecular basis of carcinogenesis.

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Analysis of Base Substitutions Induced by Ultraviolet Radiation in *Escherichia coli* [1, 2]

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ABSTRACT—A system for determining the precise specificity of mutagens operating on *Escherichia coli* has been described, and the results for UV irradiation have been presented. Attempts to correlate the base pairs preferentially mutated by UV light with pyrimidine–pyrimidine sequences are discussed.—Natl Cancer Inst Monogr 50: 115–119, 1978.

We recently developed a system that allows us to analyze a large number of point mutations in the *lacI* gene of *Escherichia coli* and to determine the precise nature of the base substitution (*1*). The *lacI* gene codes for the structure of the *lac* repressor protein (*2, 3*). Not only has the amino acid sequence of the protein been elucidated (*4*), but the entire nucleotide sequence of the *I* gene has also been determined.[4] We have taken advantage of this information to assign a large number of different nonsense sites in the *I* gene to specific codons in the corresponding message (*1, 5*). Virtually all these assignments have been verified by the nucleotide sequence of the gene. The resulting system takes advantage of 90 different nonsense sites, most of which are shown in text-figure 1. Here each position in the protein at which a nonsense mutation has been found in the corresponding portion of the message is indicated by a circle. The wild-type residue normally appearing at the point is given, except in a few instances when exact position of the nonsense site has not been determined.

The knowledge of which codon is involved in generating a nonsense mutation (amber = UAG; ochre = UAA) enables us to determine which base substitution is responsible for the conversion of a wild-type codon to either amber or ochre. Thus we can arrange each characterized nonsense site in groups according to the respective base substitution. This is done in text-figures 2 and 3 for the amber and ochre sites. All four transversions and one of the two transitions are involved in generating amber codons, although two transversions and one of the two transitions can result in ochre codons (via a single base substitution).

MATERIALS AND METHODS

All genetic techniques were described elsewhere (*1, 5*).[4]

RESULTS

To determine the specificity of a mutagen, we collected a large number of independently derived nonsense mutations and assigned them to one of the previously characterized sites (*1, 5*). We can represent the frequency of occurrence of mutations at each site on a bar graph, on which the sites have been classified according to each type of transversion or transition, as depicted in text-figures 2 and 3. The results for UV-induced mutations are shown in text-figures 4 and 5. A total of 653 nonsense mutations, each of independent origin, have been used to construct these diagrams. (Nonsense mutations constitute approximately 25% of all *I* mutations detected after UV irradiation.)

UV irradiation induces both transitions and transversions, demonstrating that there is no strict specificity for any one type of base change. For instance, some transversion sites are better induced than are those of certain transition sites. However, several transition sites seem to be particularly well induced. These represent examples of genetic "hotspots" (*6*), or highly mutable sites. The recent elucidation of the nucleotide sequence of the *I* gene makes possible the consideration of neighboring bases. For the transition sites, which are numerous, we can ask whether the mutated base pair (G:C→A:T) is part of a pyrimidine–pyrimidine sequence on one strand or the other and then compare this information with the relative rates of induction of a transition at the respective site. Thus we are asking whether sites that are part of a pyrimidine–pyrimidine sequence are more easily mutated by UV irradiation than those that are not. Table 1 shows the result of this analysis, which clearly indicates a strong correlation between potential for pyrimidine–pyrimidine dimer formation and ability to be induced by UV light. Only the transition sites that are not part of a pyrimidine–pyrimidine sequence are well induced. (A similar correlation exists for the transversion sites, although the data are not as statistically significant, since fewer sites occur in the category that is not part of a pyrimidine–pyrimidine sequence.)

An additional feature of UV irradiation revealed by this system is its tendency to induce tandem double-base changes. Approximately 2% of all of the nonsense sites found after UV irradiation have arisen from substitution of two neighboring bases. At least 15 in the collection of 90 nonsense sites result from this type of mutation; all but one of these occurs via a tandem double-base change at a pyrimidine–pyrimidine sequence. This suggests that a proportion of the misincorporations across from the pyrimidine–pyrimidine photodimers are double errors. UV-induced tandem double-base changes have been detected in yeast (*7*), and two additional examples of UV-stimulated tandem double-base changes have been

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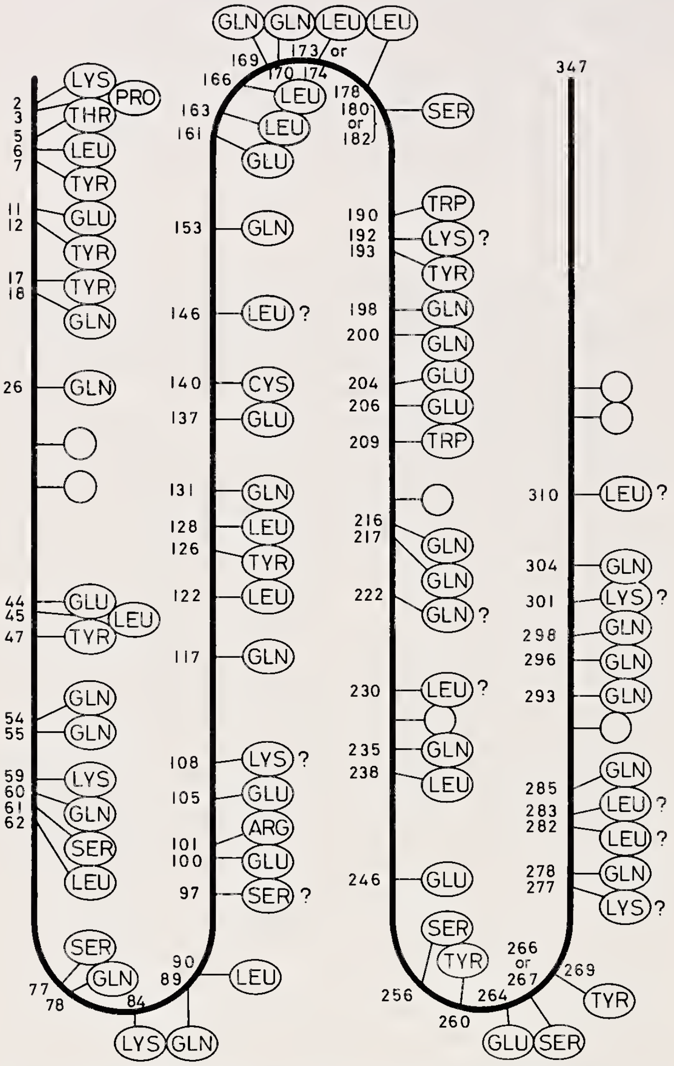
[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] Supported by grant 3.179.77 from the Swiss National Fund.

[3] Department of Molecular Biology, University of Geneva, Geneva, Switzerland.

[4] Farabaugh PJ, Calos M, Maxam A, et al: Unpublished observations.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293
GLN
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GLN
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282
LEU ?
LEU ?
278
277
GLN
LYS ?

Text-figure 1.—Sites in the *lac* repressor at which nonsense mutations in the corresponding message have been found.

Table 1.—*Frequency of G:C→A:T transitions compared with neighboring sequence*

Site[a]	Part of a pyrimidine–pyrimidine sequence on one strand[b]	No. of occurrences of G:C→A:T mutation[c]
A5	Yes	19
A6	"	15
A15	"	7
A16	"	20
A19	"	16
A21	"	5
A23	"	80
A24	"	9
A26	"	2
A33	"	36
A34	"	1
A35	"	6
013	"	3
024	"	35
027	"	49
A9	No	1
A31	"	2
010	"	2
09	"	2
011	"	1
017	"	2
021	"	1
028	"	2
029	"	2
034	"	1
035	"	2

[a] Allele numbers are those used by Coulondre and Miller *(1)*.

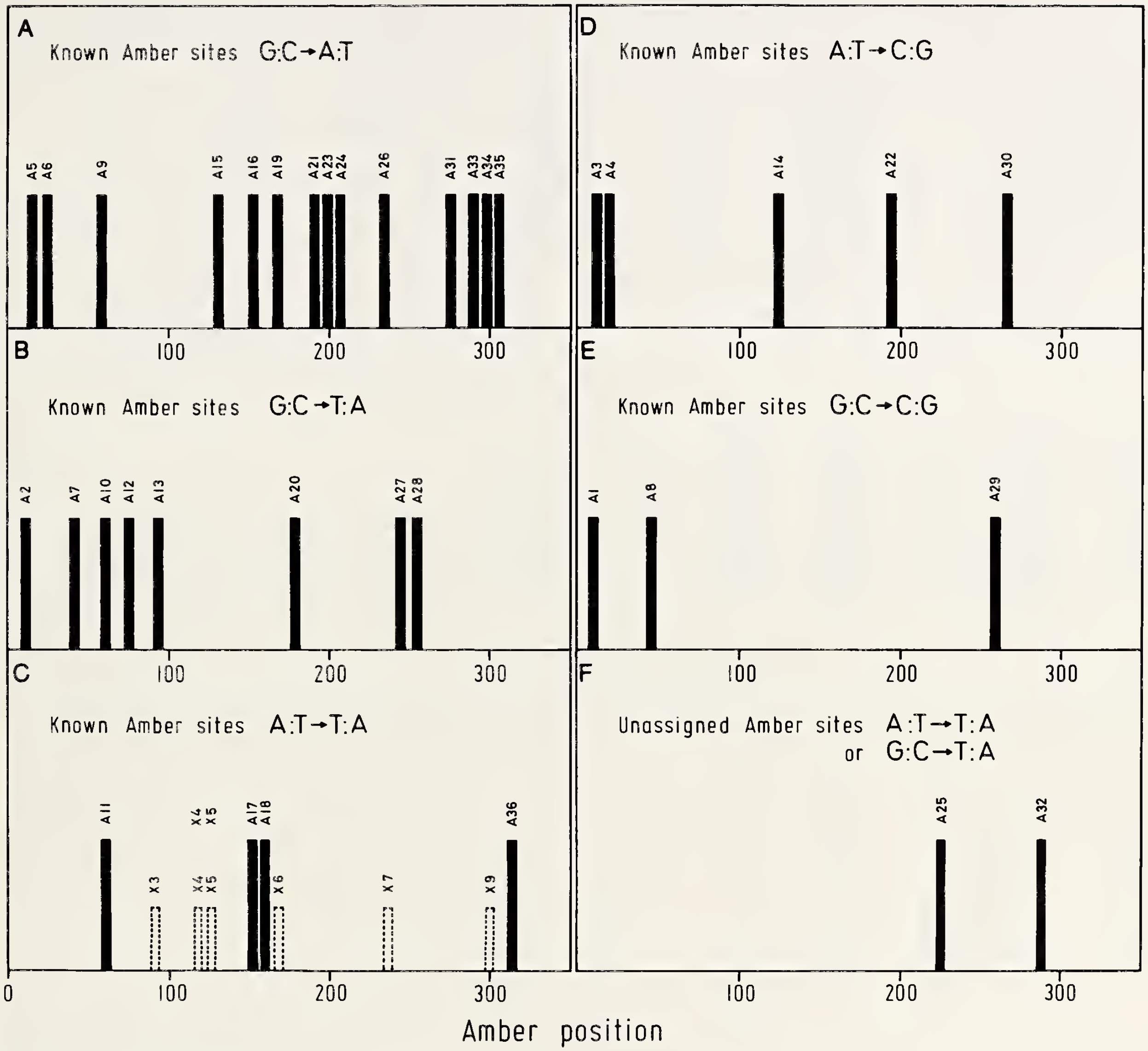
[b] Determination was made with the aid of the DNA sequence recently elucidated by Farabaugh, PJ, Calos M, Maxam A, et al. *See* footnote 4.

[c] The number of occurrences of ochre mutations was multiplied by 0.57 and then rounded to the nearest whole number to allow direct comparison with the amber mutation frequencies (*see* legend to text-fig. 5).

reported in *E. coli* [(*8*); Gilbert A, Maxam A: Unpublished observations].

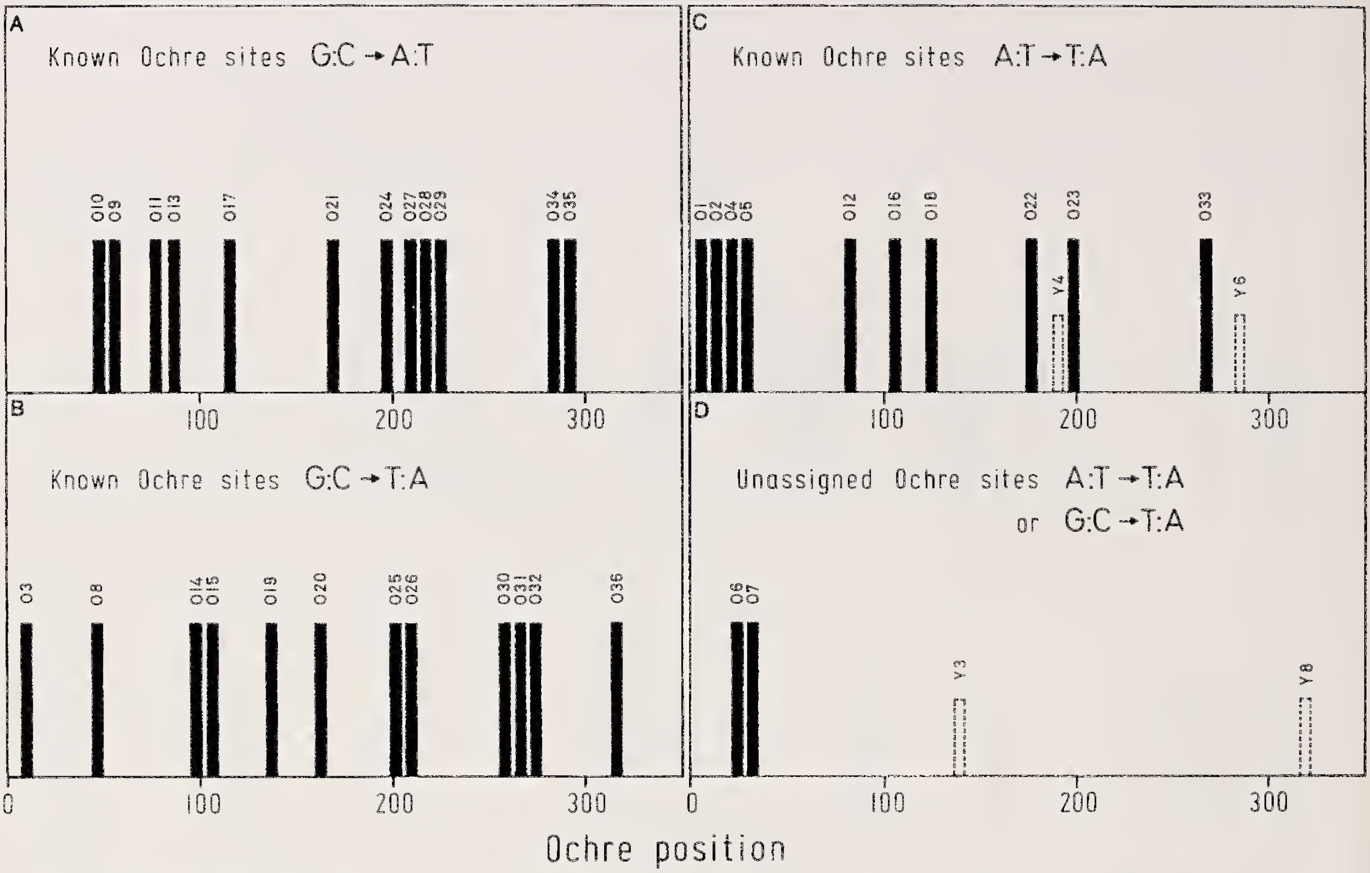
DISCUSSION

In *E. coli,* UV irradiation stimulates the induction of an error-prone repair system thought to be responsible for virtually all UV-induced mutagenesis [for review, *see* (*9*)]. The primary stimulus for this induction is the generation of pyrimidine–pyrimidine photodimers. Errors are thought to occur by repair of single-strand gaps across from the dimers, since the original base pairing specificity of the pyrimidine–pyrimidine sequence can no longer be recognized (*9*). Little direct evidence concerning the nature and neighboring base sequence of the base changes induced by UV has been presented, although previous studies have attempted to deal with this question by indirect methods (*10*). We have outlined a system capable of analyzing precisely a large number of base changes. Moreover, in this system, the surrounding

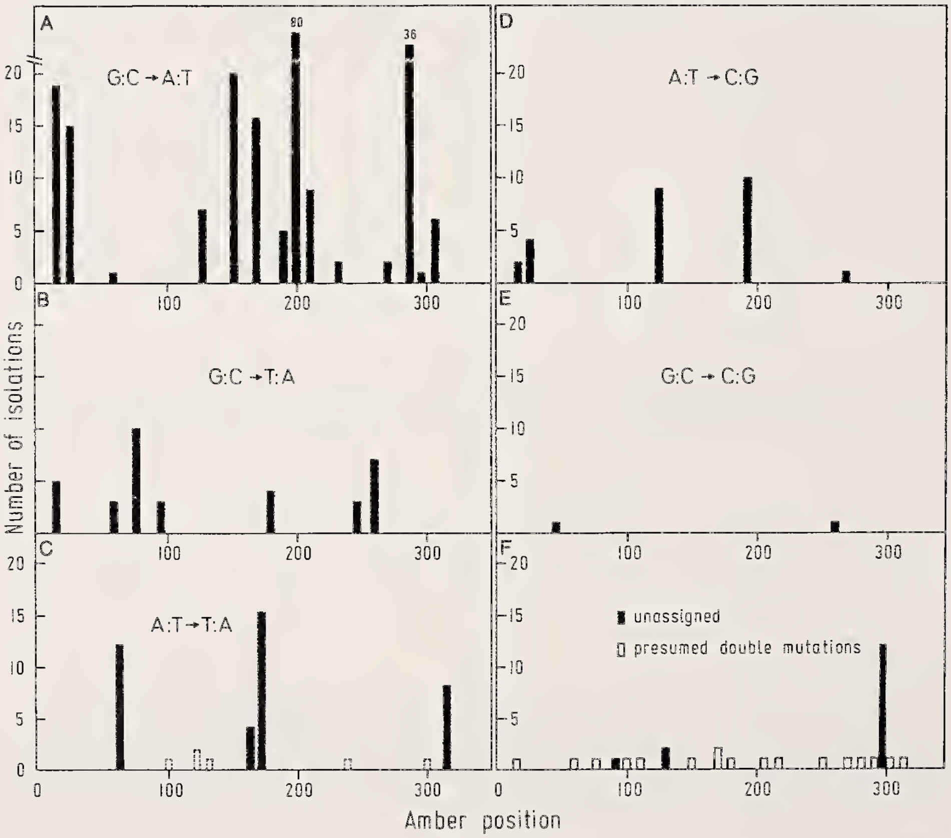


TEXT-FIGURE 2.—The known amber sites in the *lacI* gene are represented by a *bar*. The position of each *bar* in each section corresponds to the position of the nonsense site in the gene, in terms of residue number of the repressor protein. Sites have been classified according to type of base substitution required to generate the amber codon from the wild-type codon. Sites shown in *dotted lines* probably arise by tandem double-base changes (*see* text).

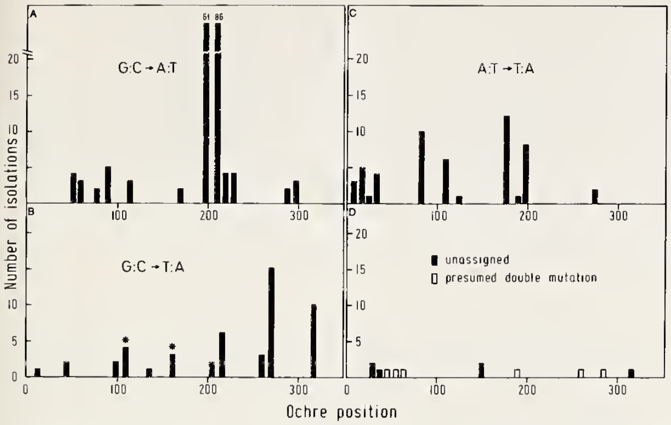
base sequence is also subject to investigation. Both transversions and transitions are stimulated by UV irradiation, and the rates of mutation can be correlated with the potential of the particular base pair to form a photodimer or at least with its presence as part of a pyrimidine–pyrimidine sequence (on one strand or the other). Also, tandem double-base changes can be detected. These appear at at least 2% of the level of single-base substitutions.



TEXT-FIGURE 3.—Known ochre sites are depicted in the same manner as shown in text-figure 2 for the amber sites.



TEXT-FIGURE 4.—Amber sites induced by UV light. *Bar* heights represent the number of independent occurrences of mutations appearing at each respective site. Nonsense mutations induced by UV light are at least 200-fold above the spontaneous background.



TEXT-FIGURE 5.—Ochre sites induced by UV light. *Bar* heights represent the number of independent occurrences of mutations appearing at each site in the total collection of UV-induced mutations. Ochre mutations generated by UV light are less frequent than amber mutations. To compare text-figures 4 and 5 directly, it is necessary to multiply each ochre bar height in text-figure 5 by 0.57.

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Mutagenesis and Cell Transformation by Ultraviolet Irradiation: Many Hypotheses for Few Results[1, 2]

Miroslav Radman,[3] Silvio Spadari,[4] *and* Giuseppe Villani[3, 5]

ABSTRACT—UV light-induced mutagenesis in bacteria is a genetically controlled process dependent on induction of some cellular functions, provoked initially by unrepaired photolesions in the DNA. Experiments on the extent and fidelity of in vitro DNA synthesis on UV-irradiated templates by bacterial and mammalian DNA polymerases suggest a crucial role for 3′ to 5′ exonuclease (proofreading) activity in UV light-induced mutagenesis. Two-stage carcinogenesis (initiation and promotion) is discussed in terms of two-stage mutagenesis (mutation fixation in the DNA and mutation expression). A unifying concept for both mutational and viral malignant transformation is proposed.—Natl Cancer Inst Monogr 50: 121–127, 1978.

UV irradiation, ionizing irradiation, and many powerful chemical mutagens and carcinogens provoke in bacteria permanent genetic changes (mutations) and temporary metabolic and phenotypic changes (SOS induction) which include induction of proviruses in lysogenic bacteria (*1–3*). SOS induction is a prerequisite for UV-induced mutagenesis in bacteria (*4*). That DNA lesions and not some other metabolic effect of UV irradiation cause SOS induction has been demonstrated by the triggering of the cellular mutagenic and repair capacity (SOS repair) in unirradiated *Escherichia coli,* which have received DNA from UV-irradiated donor cells by conjugation (*5*). Hence intact bacteria are not "competent" to perform UV mutagenesis, and that competence is temporarily induced only after damage to cellular DNA (*6*).

A certain superficial similarity exists between the phenomenologies of UV mutagenesis in bacteria and of two- (or multi-)stage carcinogenesis in animals (*3*). Chemically induced and radiation-induced tumors in animals and chemically induced and radiation-induced cell transformation in tissue culture can be produced with high frequencies as the result of a two-stage process: "initiation" by a mutagen and subsequent "promotion" by repeated exposures to tumor promoters such as phorbol esters (*7–9*). Since there is a strong qualitative (*10*) and quantitative (*11*) correlation between mutagenicity in bacteria and carcinogenicity in animals, either initiation or promotion, or both, are likely to be related to mutagenesis for a series of chemical carcinogens. Alternatively, mutagenesis could be correlated with carcinogenesis in a noncausal relationship, just as mutagenesis and prophage induction are correlated in bacteria (*4*).

We searched for answers to the following questions: 1) What are the molecular mechanisms of mutagenesis by UV light and by chemicals in bacteria and in mammalian cells? 2) If somatic mutagenesis is the basis of at least one step in malignant transformation, how could a mutation or mutations in the mammalian genome cause cell transformation with its multiple phenotypic expressions?

Therefore, our purpose is to summarize results obtained in our laboratory that we believe are relevant to the molecular mechanism of UV mutagenesis. Few illustrative results will be shown since most of them have been published elsewhere. Materials and methods for the results shown here can be found in the legends to tables and figures and, in more detail, in (*2, 12–14*).

EXTENT AND FIDELITY OF IN VITRO DNA SYNTHESIS ON UV-IRRADIATED TEMPLATES BY BACTERIAL AND MAMMALIAN DNA POLYMERASES

The finding that none of the constitutive DNA polymerases of *E. coli* can synthesize DNA past UV-induced pyrimidine dimers in ϕX174 phage DNA either in vivo (*13*) or in vitro (*2, 14*) is consistent with the lack of UV-induced mutagenesis when ϕX174 and lambda viruses are irradiated extracellularly and assayed on unirradiated bacteria (*1, 2, 4, 13*). The blockage of the in vitro DNA synthesis by *E. coli* DNA polymerase I was shown to be accompanied, and probably provoked, by an excessive turnover of nucleoside triphosphates to free nucleoside monophosphates by the polymerase-associated 3′ to 5′ exonuclease activity (*2, 14*). This exonuclease activity performs a "proofreading" or "editing" function by the preferential excision of the mismatched 3′

Abbreviations: SOS induction = cellular metabolic response to unrepaired DNA damage involving among other phenomena, induction of prophage and of SOS repair; SOS repair = inducible cellular mutagenic and repair capacity; BSA = bovine serum albumin; cpm = counts/minute; J = joule(s); AMV = avian myeloblastosis virus; α-PC = α-phosphocellulose; α-HA = α-hydroxyapatite; α-DNA cellulose; IP = inducer protease; TLCK = *N*-α-tosyl-L-lysylchloromethane; TAME = *N*-α-tosyl-L-arginyl methyl ester.

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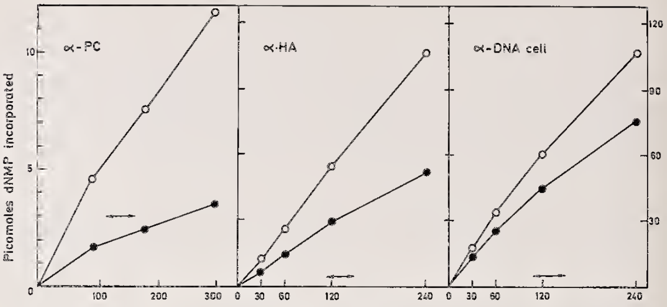
TABLE 1.—*Incorporation and turnover of complementary and noncomplementary nucleotides during the synthesis by* E. coli *DNA polymerase I on intact and UV-irradiated poly(dC) templates*

Primer:template	Complementary nucleotide Free dGMP produced (pmoles)/dGMP incorporated (pmoles)	Noncomplementary nucleotide Free dAMP produced (pmoles)/dAMP incorporated (pmoles)[a]
Oligo(dG):poly(dC) (untreated)	7.8/67.6 = 0.1[b]	0.7/0.068 = 10.3[b]
Oligo(dG):poly(dC) (UV-irradiated)	22.1/4.7 = 4.7[b]	1.0/0.0048 = 208.3[b]

[a] Stable incorporation of dAMP could not be measured directly but was extrapolated from the misincorporation frequency (dAMP incorporated vs. dGMP incorporated), which was estimated in a parallel assay and was 10^{-3}.

[b] The results shown are from 20-min incubation times in the linear part of all four reactions. Each assay (0.1 ml) contained 50 mM Tris–HCl, pH 8, 10 mM $MgCl_2$, 1 mM dithiothreitol, 100 μg BSA/ml, 3 nmoles [^{3}H]dGTP (500 cpm/pmole), 3 nmoles [α-^{32}P]dATP (500 cpm/pmole), 1 nmole poly (dC):oligo(dG), hybridized in 10:1 ratio, either intact or UV-irradiated (25,000 J/m^2), and 1 U *E. coli* DNA polymerase I (large fragment). Reactions were done at 30° C. At different times, 5-μl aliquots were withdrawn and spotted onto polyethyleneimine–cellulose plates. Chromatography was performed and respective radioactivities from regions containing the polymer, nucleoside triphosphates, and monophosphates were counted as described elsewhere *(2)*.



TEXT-FIGURE 1.—Extent of DNA synthesis, measured as picomoles of incorporated dNMP, by three fractions of calf spleen DNA polymerase α on UV-irradiated ϕX174 phage DNA. The purification procedure for this polymerase through DEAE-cellulose, phosphocellulose, hydroxyapatite, and DNA cellulose chromatography was described *(17)* except for the last step, which will be published elsewhere. Incubation mixtures (0.1 ml) contained 500 pmoles primed ϕX174 phage DNA (intact or UV-irradiated 500 J/m^2), 10 mM $MgCl_2$, 1 mM dithiothreitol, 100 μM dGTP, dCTP, dATP, and 50 μM [^{3}H]dTTP (1,500 cpm/pmole) and 0.05 U of phosphocellulose (α-PC), hydroxapatite (α-HA), or DNA-cellulose (α-DNA cell) fractions of DNA polymerase α. A parallel mixture contained 1 U of *E. coli* DNA polymerase I to indicate the level of DNA synthesis up to the first pyrimidine dimer (*horizontal arrow*). Incubation was at 37° C, and aliquots removed at indicated times were analyzed for acid-insoluble radioactivity by the Bollum filter technique [*see (2)*].

OH-terminal bases (*15*). When provoked by the 3′ to 5′ exonuclease proofreading, the turnover of nucleoside triphosphates to free monophosphates is the result of incorporation and subsequent hydrolysis of the incorporated nucleoside monophosphate. Table 1 shows that complementary and noncomplementary nucleoside triphosphates were subject to an increased turnover when the template contained cytosine dimers. The noncomplementary nucleotide was mostly turned over, which demonstrates proofreading by the polymerase.

This result suggests that the elongation of DNA chains past pyrimidine dimers is prevented by the 3′ to 5′ exonuclease activity and not by the lack of the capacity to (mis)incorporate bases opposite pyrimidine dimers. We shall refer to this dynamic blockage of DNA synthesis as "idling" of DNA polymerases. Therefore, we proposed that the inducible function(s) required for UV-mutagenesis might involve inhibition of the 3′ to 5′ exonuclease editing function of bacterial DNA polymerases (*2*).

Attribution of such a crucial role in UV mutagenesis to the polymerase-associated 3′ to 5′ exonuclease activity leads to the prediction that purified mammalian DNA polymerases α, β, and γ, which are apparently devoid of any exonuclease activity (*16*), should be able to elongate DNA chains past pyrimidine dimers. This was shown to be true for human DNA polymerases (*2*) and for AMV reverse transcriptase (*14*). Text-figure 1 shows that, unlike *E. coli* DNA polymerase I, DNA polymerase α from calf spleen can synthesize DNA past pyrimidine dimers and that this capacity improves with the degree of purification. This observation suggests the existence of a factor or factors contaminating partially purified DNA polymerase α, which inhibits DNA synthesis past pyrimidine dimers.

The improvement of in vitro DNA synthesis on UV-irradiated DNA with the increase in purification can be correlated with the loss of a contaminating 3′ to 5′ exonuclease activity (producing exclusively mononucleotides) and with the increase in the in vitro fidelity of DNA polymerase α, i.e., performing the proofreading function (table 2).

However, the alternative remains that the loss of exonuclease activity is only an indication of purification and possibly the loss of some other factor could be responsible for the phenomena in table 2. If the cause of our failure to produce UV-mutagenesis in vitro, using irradiated poly(dC) and poly(dT) homopolymers and purified bacterial DNA polymerases, was due to the 3′ to 5′ exonuclease activity (*14*), then one could expect direct in vitro mutagenesis with purified mammalian DNA polymerases and UV-irradiated templates. Table 3 shows that the phosphocellulose fractions of human DNA polymerases α, β, and γ (*17*) (although all still contaminated by the 3′ to 5′ exonuclease activity; results not shown) do show increased misincorporation frequencies during synthesis on UV-irradiated poly(dC) templates, whereas *E. coli* DNA polymerase I shows little or none. *E. coli* DNA polymerase III gave results similar to DNA polymerase I (*14*).

A SCENARIO FOR UV-MUTAGENESIS

In addition to genetic analyses of UV mutagenesis (*1–6*), this initial approach to study UV mutagenesis in vitro suggests the following scenario.

UV Mutagenesis in Bacteria

Essentially, only pyrimidine dimers that have not been eliminated by the constitutive, prereplicative excision repair

TABLE 2.—*Effect of purification of calf spleen DNA polymerase α on its fidelity and capacity to copy UV-irradiated DNA, and the loss of a 3′ to 5′ exonuclease activity*

DNA polymerase-α fraction	α-PC[a] (0.5 U)	α-HA[a] (0.5 U)	α-DNA cell[a] (0.5 U)
3′→5′ Exonuclease activity[b]			
a) Percent terminal mismatch excised from poly(dA):oligo(dT) (dC)	73	61	10
b) Percent λ-DNA degraded	39	21	11
Misincorporation dAMP/dGMP incorporated into poly (dC):oligo(dG)]	1/3,380	1/1,440	1/860
Percent inhibition of DNA synthesis by UV (500 J/m^2) of primed φX174 DNA	68	46	25

[a] Three fractions of calf spleen DNA polymerase α of increasing purity were tested for the contaminating 3′ to 5′ exonuclease activity, misincorporation ratio dAMP/dGMP on poly(dC):oligo(dG) and for inhibition of DNA synthesis by UV irradiation of primed φX174 phage DNA (*see* text-fig. 1). One unit of polymerase activity was used in a reaction mixture containing 50 mM Tris, pH 8.5, 10 mM $MgCl_2$, 1 mM dithiothreitol, 100 μg BSA/ml, and the respective substrates.

[b] 3′ to 5′ Exonuclease activity was measured as *(a)* acid-solubilization of the 3′OH terminally mismatched [^{14}C]cytosine from the poly(dA):oligo(dT) (dC) [^{14}C]substrate *(15),* and *(b)* release of nucleoside monophosphates from the native DNA of phage λ. Details will be published (Spadari S, Villani G, Radman M: Submitted for publication).

before being reached by the DNA replication machinery are potentially mutagenic lesions (*1*). Bacterial DNA replication machinery cannot elongate nascent DNA chains past pyrimidine dimers, probably because of polymerase idling due to associated 3′ to 5′ exonuclease proofreading activity. However, DNA replication continues by reinitiation beyond pyrimidine dimers, leaving gaps in the newly synthesized strands opposite dimers in the parental strands (*18*), which are repaired in *E. coli* by recombinational exchanges (*18*) in an essentially error-free fashion (*1*). Pyrimidine dimers causing gaps that cannot be repaired by recombination probably also provoke SOS induction due to the continuous idling of DNA polymerases. A change in the nucleoside monophosphate to triphosphate ratios in the intracellular pool (as a consequence of polymerase idling and/or DNA degradation) could lead to the activation of the IP cleaving phage λ repressor (*19*) and perhaps some cellular repressors of the "SOS functions" (*1, 2, 20*). A possible result of SOS induction might be a modification of the DNA replication machinery, such as the inhibition of the 3′ to 5′ exonuclease activity of DNA polymerases (*2*), which should result in both DNA synthesis past pyrimidine dimers (increased survival) and decreased replicational fidelity (mutagenesis).

UV Mutagenesis in Mammalian Cells

The prereplicative excision repair of pyrimidine dimers that eliminates potentially mutagenic lesions is deficient in the fibroblasts of some patients with xeroderma pigmentosum, and this leads to a "dose reduction" for UV mutagenesis (*21*). Several phenomena in radiation biology are consistent with the existence of an inducible DNA repair system in mammalian cells (*3*), and in particular the increased survival of irradiated viruses when assayed on irradiated host cells (*22*). This increase in survival raises the possibility that mam-

TABLE 3.—*Fidelity of human (HeLa) DNA polymerases α, β, and γ and of* E. coli *DNA polymerase I copying intact and UV-irradiated poly(dC) template*[a]

Primer:template (dG_{12}:dC_n)	DNA polymerase	Complementary nucleotide, pmoles dGMP incorporated	Noncomplementary nucleotide, pmoles dAMP incorporated	Error frequency
Intact	α	81.79	0.0232	1/3,379
UV-irradiated		58.35	0.0244	1/2,391
Intact	β	270	0.0307	1/8,794
UV-irradiated		97	0.0211	1/4,597
Intact	γ	228	0.1086	1/2,103
UV-irradiated		188.84	0.1084	1/1,742
Intact	*E. coli* I	1,747	0.709	1/2,464
UV-irradiated		1,112	0.502	1/2,215

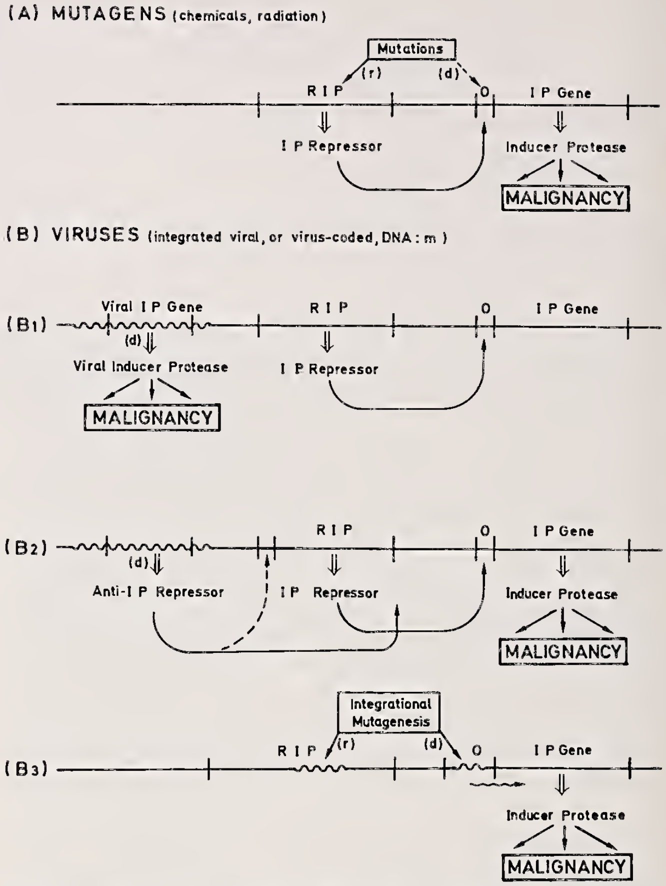
[a] All reaction mixtures (0.1 ml) contained 50 mM Tris, pH 8.3, 1 mM dithiothreitol, 10 mM $MgCl_2$, 100 μg BSA/ml, 20 μM [^{3}H]dGTP (200 cpm/pmole), 20 μM [α^{32}P]dATP (10,000 cpm/pmole), and 50 μM dG_{12}:dC_n (Collaborative Research) hybridized in the ratio of 1:3. To each assay we added 10 μl DNA polymerase α (8 U/ml), 15 μl DNA polymerase β (60 U/ml), 10 μl DNA polymerase γ (31 U/ml), and 0.5 U *E. coli* DNA polymerase I. Reactions were saturated at 36° C for 1 hr, stopped by the addition of 0.2 ml 10% perchloric acid, and pelleted by centrifugation. To eliminate excess acid-soluble radioactivity, pellets were resuspended in 0.2 N NaOH, reprecipitated with perchloric acid twice, and finally spotted onto GF/C glass fiber filters; radioactivity was counted as described *(2).* UV irradiation (4,500 J/m^2) of the dG_{12}:dC_n was performed in 50 mM Tris, pH 8, and 20 mM KCl, on ice, in a thin layer as described *(2).* The blank reaction mixtures contained all reagents, and respective enzymes except dG_{12}:dC_n and their radioactivities, although negligible, were subtracted from those of complete reaction mixtures. The incorporation of the noncomplementary dAMP depended on the presence of complementary nucleoside triphosphate (dGTP).

malian cells may also be mutable by the inducible SOS repair. The efficient in vitro DNA synthesis by highly purified DNA polymerase α is unlike in vivo DNA synthesis in mammalian cells, in which all, or nearly all, pyrimidine dimers cause gaps in the newly synthesized DNA (*23*). This discrepancy could be accounted for by our preliminary evidence (table 2) that a 3′ to 5′ exonuclease may be associated with, but is not an integral part of, mammalian DNA polymerase α. Its function may be analogous to bacterial polymerase-associated 3′ to 5′ exonuclease in increasing the fidelity and preventing DNA chain elongation past pyrimidine dimers (table 2). In this instance, the inactivation or dissociation from polymerase of the 3′ to 5′ exonuclease may be required for UV mutagenesis in mammalian cells like that proposed for bacterial mutagenesis.

A UNIFYING CONCEPT FOR MALIGNANT TRANSFORMATION INDUCED BY MUTAGENS AND VIRUSES

As mentioned previously, correlations between mutagenicity and carcinogenicity do not necessarily imply causality between the two phenomena. In particular, we know that mutagens can also exert epigenetic effects (SOS induction) in bacteria which, among other effects, are also prerequisites for mutagenesis. Therefore, we can only speculate about possible genetic mechanisms of malignant transformation with the aim of producing testable hypotheses to provoke experimental tests. Text-figure 2 illustrates a unified working hypothesis for mutational- and virus-induced cancer. This hypothesis was inspired by the postulated mechanism of SOS induction in *E. coli* (*1–4*) that has been incorporated into the concept of two-stage carcinogenesis, i.e., "initiation" by mutagens and subsequent "promotion" by essentially noncarcinogenic promoters (*7, 24*).

The basic assumptions of the hypothesis are: 1) No introduction of new genetic information is required for malignancy. 2) Since promoted cells show most phenotypes of the transformed cells (*25, 26*), the transformed state is being considered as a state of permanent promotion. 3) Temporary and permanent promotion (i.e., transformation) are initially caused by an intracellular IP, which might be a plasminogen activator [for further discussion, *see* (*2*)]. 4) Multiple phenotypic expressions of transformed cells are due to activation of many genes and/or gene products by the IP, which is supposed either to cleave or activate the cleavage of genetic repressors, or to activate inert gene products by limited proteolysis. 5) The IP may be the plasminogen activator temporarily induced by tumor promoters, such as phorbol esters (*25*). 6) This temporary induction of the IP during promotion can occur by inactivation of the repressor of the IP by the direct binding of phorbol esters to the repressor. 7) Permanent induction of the *IP* gene can occur by inactivation of its repressor either mutationally (text-fig. 2, schemes A and B3) or by a viral gene product (schemes B1, B2, B3). A list of references and source information possibly relevant to the proposed models is presented below.



TEXT-FIGURE 2.—A unified working hypothesis for malignant transformation by chemicals, radiation, and viruses. Transformation is considered as permanent promotion through permanent activation of the gene coding for IP. IP is supposed to cleave (or activate the cleavage of) cellular genetic repressors and/or to activate inert zymogens by limited proteolysis. All the phenotypes, including the active mitotic machinery, could be the consequence of the initial action of the IP. A) Mutagens could activate the *IP* gene by mutations in the gene coding for the repressor (RIP) of the *IP* gene. Such a malignant mutation is expected to be recessive (r). Possible mutations in the operater region (o) are expected to be dominant (d). B) Viruses could either code for their viral IP and transform independently of the cell functions (B1) or they could code for an anti-repressor of the cellular IP repressor and hence keep the *IP* gene induced as long as viral anti-repressor is produced (B2 model), or the integration of viral DNA into specific regions of the IP operon (B3 models) could cause activation of the *IP* gene. B3 model has two versions: The first is recessive integrational mutagenesis [B3 (r) model], and the second is a dominant *cis*-activation of the IP operon by the activation of transcription from the viral DNA into the IP operon, requiring permanent expression of a viral gene product [B3(d) model]. *See* text for further discussion.

REFERENCE MATERIAL RELEVANT TO THE PREMISES OF THE HYPOTHESIS

1) Trypsin-like proteases are likely mitotic inducers in normal cells. Trypsin activates G1-blocked isolated nuclei (*27*) and penetrates cell and nuclear membranes (*28*). Activation of chick erythrocyte nuclei by active HeLa nuclei in

cell hybrids is decreased by inhibitors of the trypsin-like proteases, TLCK and TAME (*29*). The mitotic activity of the HeLa nucleus can be suppressed by an excess of inactive chick erythrocyte nuclei in heterokaryons, and this is facilitated by TLCK and TAME (*30*).

2) Tumor promoters (phorbol esters) are powerful inducers of a temporary production of a plasminogen activator by normal cells in vivo and in vitro [(*25, 26, 31*) and references therein].

3) Permanent production of a plasminogen activator is correlated with the transformed state, independent of the transforming agent (*32, 33*).

4) Some natural and synthetic inhibitors of trypsin-like proteases have been shown to inhibit tumorigenesis in experimental initiation–promotion carcinogenesis in mice (*31*) and, curiously enough, also to suppress UV mutagenesis and UV induction of prophage λ in *E. coli* (*2, 20*).

5) The appearance of plasminogen activator in 2 and 3 requires active RNA and protein synthesis (*25, 32, 33*).

6) Chemically induced cell transformation may result from the mutagenesis of a single locus (*34, 35*). (Compare with text-fig. 2A.)

7) Mutagen-induced and spontaneous transformations appear recessive in cell hybrids between malignant and normal cells as long as a specific normal chromosome or chromosomes are present in the heterokaryon [(*36, 37*) and references therein]. In other words, cancer can be suppressed but not "erased," since it reappears following chromosome loss from heterokaryons (*36*). (Compare with text-fig. 2A.)

8) Whatever the transforming agent, mutagen or virus, various tumor cells do not complement in cell hybrids, i.e., heterokaryons remain malignant (*38*).

9) The transformed character of cells recently transformed by a tumor virus appears dominant in cell hybrids (*39, 40*). (Compare with text-fig. 2 B1, B2, B3.)

10) In virus-transformed cells, the plasminogen activator is a cell and not a virus type (*32, 33*). This discourages the B1 model if the plasminogen activator is the IP.

11) Continuous production of an active viral early gene product (T-antigen) is necessary to maintain virus-induced transformation (*41*). This discourages model B3(r).

12) The phenomenon of abortive, transitory transformation by simian virus 40 and polyoma viruses (*42, 43*) indicates that stable integration of viral DNA into host genome may not be necessary for the establishment of transformation but rather for its maintenance. This further discourages B3(r) and B3(d) models. In conclusion, some version of B2 is our preferred model for viral transformation. T-antigen, or a part of it, could be the anti-repressor of the *IP* gene (text-fig. 2, B2, *solid line*) or the repressor of its repressor (text-fig. 2, B2, *broken line*). B3(d) model still remains an attractive alternative to the B2 model. [Compare with (*39*).]

A COMMENT

The proposed model (text-fig. 2) was chosen exclusively for its minimal complexity and not as an attempt to account for the genetic mechanisms of various types of malignant transformation. It is meant to illustrate the basic idea rather than to insist on the precise mechanism of induction or activation of the IP. Ideas for promotion and transformation were borrowed from microbial genetics, e.g., induction of the IP in promotion (*2*) is like induction of the *lac* operon (*44*), i.e., through reversible inactivation of the repressor by the binding of effector molecules. Transformation is like induction of prophage λ and the pleiotropic SOS induction (*1, 2*), i.e., through proteolytic cleavage (*19*) of repressors (*1, 2, 20*); an anti-repressor protein is known in phage P22 that inactivates P22 repressor as well as the repressors of several unrelated phages including λ (*45*); B3(r) and B3(d) models were obviously inspired by the biology of bacteriophage μ and insertion sequences in *E. coli.*

The malignancy in the proposed model should persist only as long as active IP is produced and as long as the substrates (repressors and/or inert precursor proteins) remain susceptible to its action. Therefore, several mechanisms of suppression of cancer, which can be either pleiotropic (i.e., for all phenotypes of transformed cells) or partial (i.e., for one or few phenotypes only), are inherent to the model. These possibilities will be published elsewhere.

MUTATION FIXATION, MUTATION EXPRESSION, AND PROMOTION

Mutation fixation following UV irradiation of bacteria takes place during the first postirradiation DNA replication round (*1*). The fixation of X-ray-induced malignant transformation in cell culture requires two (*46*) or more (*47*) postirradiation generations. However, the problem of the expression of recessive mutations is expected to be more complex in diploid mammalian cells than in bacteria. It is likely that two events are required for the appearance of mutant (or transformed) mammalian cells: the mutation fixation in the DNA and a segregational event. If the mutagen-induced cell transformation is the consequence of a recessive mutation (*37*), this should require functional homozygosity for its expression in diploid somatic cells. Homozygosity can be brought about either by mitotic recombination (exchanges which occur between mutant and wild-type chromatids, to be segregated into different daughter cells in the region between the mutation and the centromere) following S-phase, or by chromosome loss by nondisjunction, or chromosomal rearrangements (*48*).

Since tumor promoters act as general inducers (*26*) and also increase the yield or recovery of mutagen-initiated cancer (*7, 8*), three testable hypotheses for promotion in somatic mutation theories of cancer can be envisaged. 1) Promoters could induce mitotic recombination or chromosome loss and/or rearrangements, 2) promotion could be mutation expression through derepression of otherwise silent, mutated genes (*49*). 3) Promoters could act as co-mutagens by amplifying the mutagenic effect of unrepaired DNA lesions, analogous to SOS repair in bacteria (*2, 3*). The problem of mutation expression remains the same in 3 as in 1 or 2. Experiments are being initiated in our laboratory to distinguish between 1, 2, and 3.

Most of the preceeding general discussion on the mechanisms of mutation fixation and expression and on the nature of malignant change applies par excellence to UV irradiation. At effective mutagenic and carcinogenic doses, UV light almost exclusively causes damage to nucleic acids. Furthermore, the deficiency in a repair system which removes UV-induced pyrimidine dimers from the cellular DNA is accompanied by the mutation- and cancer-proneness to UV light [(*21*) and references therein]. UV irradiation has acted as a complete carcinogen in humans, animals (*50*), and in cell culture (*51*), and as an initiator when the transforming effect is amplified by or requires a tumor promoter [(*8*) and references therein]. Since high doses of an initiator also provoke the metabolic effects of promotion (*52*), the complete carcinogenesis by high doses of initiators can be interpreted in terms of the two-stage model (*2, 3*).

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Photoreactivation: Evaluation of Pyrimidine Dimers in Ultraviolet Radiation-Induced Cell Transformation[1, 2]

Betsy M. Sutherland[3, 4]

ABSTRACT—The PRE repairs DNA by the specific and exclusive monomerization of pyrimidine dimers. Photoreactivation thus provides an analytical test for determining the role of pyrimidine dimers in UV damage to biologic systems. We have shown the first UV transformation of cultured human cells (judged by ability to grow in soft agar). Preliminary results indicate that 1) lesions leading to cell transformation are subject to photoreactivation and 2) pyrimidine dimers may well be important in solar carcinogenesis.—Natl Cancer Inst Monogr 50: 129–132, 1978.

UV light produces mutations and death in prokaryotes and simple eukaryotes (*1*) and skin cancer in man (*2*). For simpler organisms, we have good biologic, biochemical, and biophysical evidence that cyclobutyl pyrimidine dimers in DNA are a major cause of UV-induced cell damage (*1*). Whether the same is true for skin cancer produced by solar exposure or whether any one type of chemical lesion can be so pinpointed is a question of considerable interest.

PHOTOREACTIVATION: AN ANALYTICAL TOOL

A principal tool in determining the biologic importance of dimers has been enzymatic photoreactivation (*1, 3*). The PRE repairs UV damage to DNA by the specific and exclusive monomerization of pyrimidine dimers (*4–6*). Text-figure 1 shows that the enzyme binds to a dimer-containing region of DNA, and on the absorption of a photon (300–600 nm), it monomerizes the dimer and restores biologic activity to the DNA (*4–7*). Since the PRE acts only on pyrimidine dimers, the photoreactivation test can be used to evaluate the role of dimers in the induction of biologic damage: If UV-induced potential biologic damage could be reversed by true enzymatic photoreactivation, pyrimidine dimers were a major cause of that damage.

Although this test would seem ideally suited to probe UV-induced damage in mammalian cells, several obstacles have impeded its use. The most important problem was that the enzyme was thought to be absent in mammalian cells! [*See* (*8–11*).] Even after the demonstration that the enzyme was present in these cells, questions of action and specificity of the mammalian PRE remained. Thus we can set up the following conditions that must be fulfilled before photoreactivation can be used as a test of dimers producing UV damage in mammalian cells. The enzyme must 1) be present in the cells; 2) act on pyrimidine dimers in DNA; 3) act specifically on dimers; 4) have access to cellular DNA and thus must be able to monomerize dimers in intact cells; and 5) be able to mediate restoration of biologic function. We shall examine the evidence pertinent to each of these points, with special emphasis on areas in which further experimentation is critically required.

Presence of the Enzyme

PRE activity has been found in human leukocytes (*12*), human and murine fibroblasts (*13*), bovine bone marrow (*13*), feline corneal cells (*14*), human placenta (Farland WH, Sutherland BM: Unpublished results), and potoroo and ox corneal cells (*14*).

The enzyme from human leukocytes was purified to apparent homogeneity (*12*); the behavior of the enzyme during purification as well as its molecular weight and isoelectric pH indicate its similarity to PRE from other organisms (*11*). It differs in action spectrum and pH optimum from those from yeast and *Escherichia coli* (*12, 15*). However, a considerable degree of diversity in peak of action spectra and pH optimum occurs in PRE from other species (*11*).

The enzyme is present at different levels in various cell types within one organism (*13*), at different ages or developmental stages within one species (*13*), and under different culture conditions of the same cell type (*16*). This variability may reflect regulation of DNA repair enzyme levels, although the identity of the effectors is not yet clear. Since many factors can affect PRE levels in cells and in intact organisms, it is clear that the first step must be a determination that the enzyme is present.

Action of the Mammalian PRE on Dimers

Use of the photoreactivating activity in an evaluation of the function of dimers requires that the enzyme actually be able to monomerize dimers. This demonstration can be done in two stages: The enzyme can cause dimer disappearance in a light-dependent reaction, and the dimers are converted to two monomer pyrimidines. For the human PRE, the first test of its action on dimers in DNA examined the action of the

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Abbreviation: PRE = photoreactivating enzyme(s).

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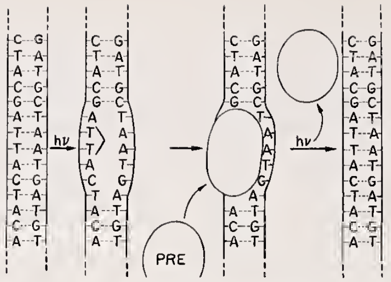
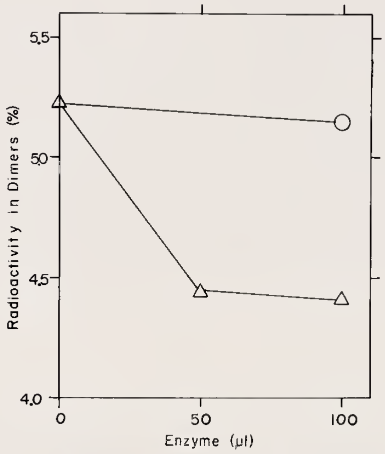
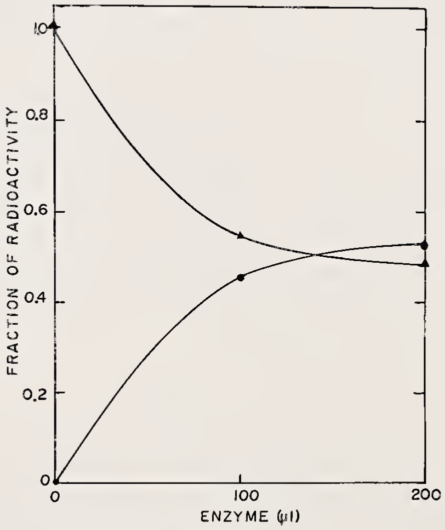


Text-figure 1.—Action of the PRE on DNA.



Text-figure 2.—Photoreactivation of pyrimidine dimers in purified [^{3}H]thymine-labeled T7 DNA by a PRE from human cells. ○ = samples exposed to photoreactivating light; △ = samples kept in the dark.



Text-figure 3.—Conversion of U[ ]U and U[ ]T dimers to the corresponding monomer pyrimidines by human PRE. ▲ = dimer content of DNA; ● = monomers appearing from enzyme action on dimers.

human leukocyte enzyme on [^{3}H]thymine-labeled, UV-irradiated T7 DNA (*12*). In the presence of visible light, the enzyme mediated the disappearance of dimers from the DNA, whereas no change in dimer content of the DNA occurred in the absence of light (text-fig. 2).

Such a test indicates that the enzyme acts on dimers, but it does not provide evidence for the conversion of dimers to monomers. A demonstration of this type is provided by examination of the action of the enzyme on [^{3}H]cytosine-labeled, UV-irradiated DNA that has been heated to deaminate all cytosine-containing dimers to uracil-containing dimers (*17*). The human leukocyte enzyme converts U[ ]U and U[ ]T dimers stoichiometrically to monomer pyrimidines as illustrated in text-figure 3 (*13*). PRE from other mammalian species have not yet been examined in such tests; clearly, both assays are important before the biologic applications of the enzymes can be valid.

Specificity of the Mammalian Enzyme for Dimers

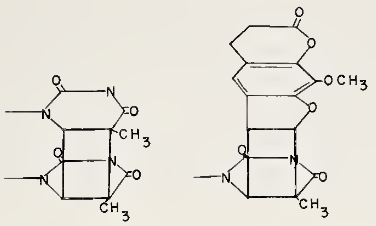
The photoreactivation test relies on the specific action of the PRE on pyrimidine dimers in DNA. Although such specificity is often attributed to all photoreactivating activities, experimental evidence exists only for the PRE from bakers' yeast (*5*). This is an important area in acute need of research.

In examining other possible substrates for PRE, we considered that photoproducts with structures similar to that of the cyclobutyl ring of the dimer might be likely substrates for the enzyme. Text-figure 4 shows the structure of a dimer and one configuration of a psoralen–DNA adduct (*18*). Thus we examined the ability of the *E. coli* and human PRE to liberate free psoralen from either monoadducts of psoralen and DNA or from psoralen cross-linked to DNA. Preliminary results indicate that neither enzyme liberated free drug from the DNA, whether the complex had been in the monoadduct or biadduct form (Sutherland BM, Farland WH, Song P-S: Unpublished results). Since these enzymes do not seem to act on nondimer photoproducts, even though they had similar structures (the cyclobutyl ring), it is not likely that less structurally related photoproducts could be repaired. Nevertheless, examination of the action of mammalian PRE on additional substrates is clearly an important area of investigation.

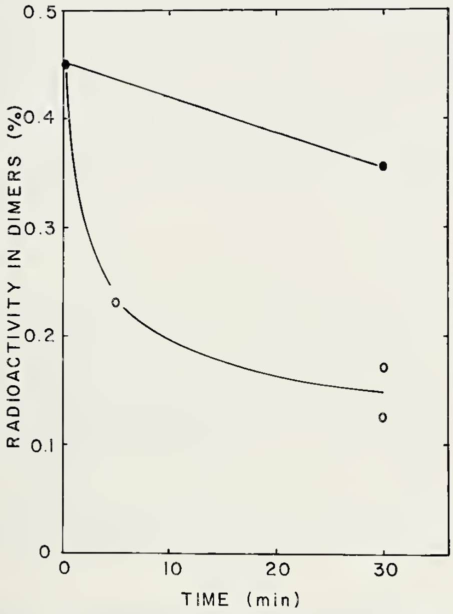
Action of the Mammalian Enzyme on Cellular DNA

Even if a PRE is present in a cell, it might not have access to cellular DNA and thus would be unable to mediate biologic repair. This action has been reported by Mortelmans et al. (*19*), who found that xeroderma pigmentosum cells contained an endonucleolytic activity that could nick UV-irradiated purified DNA, although it was not active on UV-irradiated chromatin (*19*). Thus a demonstration of the cellular action of the enzyme is essential to an evaluation of its function. The PRE from human fibroblasts can act on dimers in cellular DNA and cause their disappearance from the DNA in the light but not in the dark as illustrated in text-figure 5 (*13*). Both normal and xeroderma fibroblasts can mediate cellular dimer photoreactivation (*13, 20*).

Are all dimers available to action by the PRE? In cellular photoreactivation studies, about 70% of the total dimers were removed by the enzyme. Whether the incomplete



TEXT-FIGURE 4.—The structure of a thymine–thymine cyclobutyl dimer *(left)* and a thymine-8-methoxypsoralen adduct *(right).*



TEXT-FIGURE 5.—Photoreactivation of dimers in human fibroblasts (○ = cells exposed to photoreactivating light after UV; ● = cells kept in the dark after UV radiation.)

photoreactivation represents competition with other dimer-binding proteins, which could not be displaced by the enzyme or heterogeneity in dimers as substrate for the enzyme either by location in a particular DNA sequence or by time, is not yet clear. However, since no clear demonstration has been made of the action of the enzyme in removing all the dimers from purified DNA in vitro, this question should be resolved before consideration of possible cellular competitors or complications.

Biologic Effect of Mammalian PRE

Finally, if the mammalian PRE is to be useful in a biologic test, it must be able to restore biologic activity to UV-irradiated DNA. Three lines of evidence indicate that this occurs in human PRE. Harm (*14*) has shown that extracts of human cells restore biologic activity to UV-irradiated, bacterial transforming DNA. Wagner et al. (*21*) found that the plaque-forming ability of UV-irradiated herpes simplex DNA could be photoreactivated in human fibroblasts . That UV-induced DNA synthesis depression could be photoreactivated in human cells has been demonstrated (*22*). At present, no assessment of photoreactivation of colony-formation or mutation rates has been made in cells that contain PRE activity; clearly, such experiments are essential for the evaluation of the role of dimers in killing and mutation in human cells.

UV DAMAGE IN MAMMALIAN SYSTEMS

Now that we have criteria for selecting and testing cells and their photoreactivating abilities, what biologic damage shall we examine? First we might consider UV-induced skin damage or tumor formation in the intact animal. Despite the obvious appeal of studying the damage in situ, possible metabolic responses of the intact organism to photoreactivating light must be excluded before recovery phenomena can be attributed to photoreactivation.

UV Damage in Intact Organisms

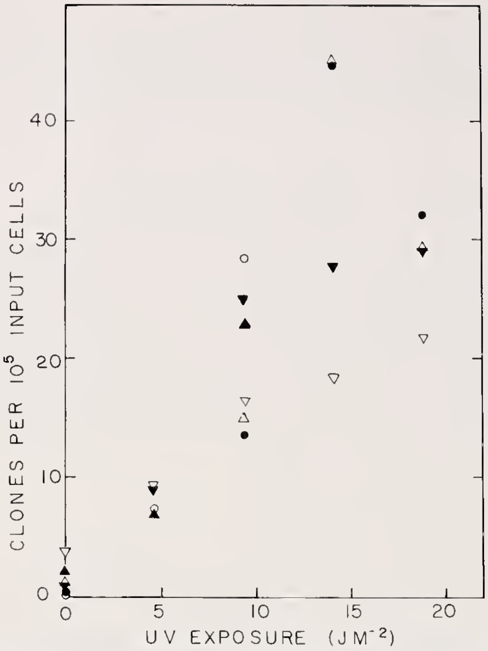
Reports of photoreactivation of UV damage in intact mammals include those of Kelner and Taft (*23*), who examined photoreactivability of UV-induced tumors in mice (although their data indicate positive results, the authors point out that the limited number of mice in each test group precluded more than tentative conclusions), and van der Leun and Stoop (*24*), who reported photoreactivation of UV-induced human erythema. Perhaps because of consideration of such possible complications, investigators have recently used cells in culture rather than the intact animal.

UV Transformation in Cultured Mouse Cells

The first requirement is a model system for UV carcinogenesis. Mondal et al. (*25*) reported a promising system in the mouse cell C3H/10T1/2. After treatment with tetradecanoyl phorbol acetate, UV exposure induced transformation of the cells. Shortly thereafter, Chan and Little (*26*) detected the induction of transformation by UV in clone 8 of the same mouse cell line. Although PRE has been found in mouse 3T3 lines, the level varied with origin of the line and with the state of growth in the cells (*13*). The levels of PRE in the mouse cells used by Mondal et al. and by Chan and Little are not known.

UV Transformation of Human Fibroblasts

To study UV-induced transformation, we selected human fibroblasts. These cells contain PRE (*20*), which can act on dimers and convert them to monomers (*27*). The human PRE seems to act specifically on dimers, and it does have access to them in cellular DNA, as shown by cellular dimer monomerization. We exposed these cells to UV radiation from a low pressure mercury lamp, allowed them to grow, and tested for their ability to grow in soft agar. [Shin et al. (*28*) have shown that tumorigenicity correlates with the ability to grow in soft agar; other criteria show poorer correlation with tumor-forming ability]. The results of 6 independent experiments on UV induction of the ability of cells to grow in soft agar are given in text-figure 6. Increasing exposure to UV raises the number of colonies observed on the agar plate. Does exposure to photoreactivating light decrease this level? Preliminary experiments prove that exposure to photoreac-



TEXT-FIGURE 6.—Effect of UV exposure on the ability of human fibroblasts to grow in soft agar.

tivating light yields a substantial decrease in the number of colonies per plate.

The development of a system for examining UV transformation in cultured human cells leads us to a multitude of experiments: 1) The properties and tumorigenicity of the transformed cells can be examined; 2) we can ask whether the UV transformation process results from lesions in the DNA, in a protein (perhaps a membrane component?), or both, or neither; and 3) we can examine the action spectrum for photoreactivation to see if it corresponds to that for enzymatic dimer photoreactivation (*27*). These experiments should allow us to evaluate directly the role of pyrimidine dimers in the transformation by UV of human cells.

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Inhibition of Skin Carcinogenesis In Vivo by Caffeine and Other Agents[1, 2]

Francois Zajdela[3] *and* Raymond Latarjet[4, 5]

ABSTRACT—The induction of skin cancer in mice of the Swiss (Carshalton) strain, by repeated irradiation with UV-light, was strikingly reduced by the local application of caffeine prior to each exposure. Theophylline displayed the same activity. These two substances have been selected as probable inhibitors of error-prone, postreplicative DNA repair. Conversely, reductone and chloroquine, which are considered as inhibitors of the error-free, prereplicative excision repair, did not modify the incidence of the tumors. Special emphasis has been given to the histologic behavior of radiolabeled caffeine in the normal and UV-irradiated epidermis of the mouse in vivo and to the ability of mouse epidermal cells in vitro to repair DNA after UV irradiation.—Natl Cancer Inst Monogr 50: 133–140, 1978.

The experiments reported here were suggested in 1969 by the new concept of error-prone DNA repair as a mutagenic event [for a review, see (*1*)]. Despite the fact that such a process had not been observed in mammalian cells, a study was undertaken on the effect of a postreplication repair inhibitor on a carcinogenic process. Caffeine was selected.

In referring to the production of malignant tumors in the animal, we reduced the questions concerning the relevance of the findings, but their interpretation resulted in maximum complexity.

Carcinogenesis by polycyclic hydrocarbons was first studied by Dr. Zajdela from 1969 to 1971.

The two-stage carcinogenesis method was used on skin on the back of 420 Swiss (Carshalton) mice during the resting stage of the hair cycle. During the 10 days after initiation with DMBA, a solution of caffeine in acetone was applied daily. This caffeine treatment reduced the incidence of tumors by about 30% after 320 days. This result was not published because caffeine is known to increase the solubilization of hydrocarbons in water. The observed decrease in tumor incidence could be due to an elution phenomenon accelerating the elimination of the carcinogen from the target cells, thus decreasing the time of action.[6] Consequently, we were led to use UV light as the carcinogen because the quantitation is precise. We began that new work in 1971.

UV was used at a dose that yielded tumors on both ears of about 90% of the mice (*see* "Materials and Methods"). Additional treatment with caffeine or theophylline gave some clear-cut results (*2, 3*); these substances, considered inhibitors of postreplication DNA repair, significantly and reproducibly reduced tumor incidence. Our results have been confirmed in several in vivo and in vitro systems (*4–7*).

These results have raised several problems:

1) Does caffeine reach the target epidermal cells when painted on the surface of the skin? We studied that question by radioautography and with tritiated caffeine.

2) The available data concerning the DNA repair mechanisms in rodents (particularly in mice) are contradictory. By radioautography, we studied the unscheduled DNA synthesis in irradiated mouse cells, i.e., the cells of an established line of mouse origin and the epidermal cells in primary culture from our mice. This method only gives access to identification of prereplication repair, which is free of error. According to our working hypothesis, caffeine should have no effect on it.

3) Instead of caffeine, use of other substances that are likely to inhibit only excision repair was indicated. If our hypothesis is correct, these substances should not affect tumor incidence.

MATERIAL AND METHODS

Animals.—We used specific pathogen-free, 10- to 12-week-old mice of the nonhomozygous Swiss strain (Carshalton). None of 1,300 control 18-month-old animals showed a spontaneous tumor on the ears. We used females only to avoid mortality by fighting. They received Millipore-filtered water and complete diets (autoclave sterilized) ad libitum. They were housed in rooms equipped to receive filtered sterile air. About 1,000 mice were treated.

Irradiation.—Each animal was immobilized in a compartment of a square cage covered with a fine mesh screen that cast practically no shadows. Isolation of the animals prevented them from shielding one another from the rays or licking each other's ears during exposure. The shape of the lamp with its reflector and the shape of the cage assured an almost uniform dose distribution, which varied only by 10% from the center to the edge of the field. Moreover, the distribution of the mice among the compartments of the cage varied randomly from day to day.

Abbreviations: DMBA = 7,12-dimethylbenz[*a*]anthracene; PBS = phosphate-buffered saline.

[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] Supported by the Comite d'Etude des Vols Stratosphériques.

[3] Institut National de la Santé et de la Recherche Médicale, Batiment 110, Institut du Radium, 91405 Orsay, France.

[4] Fondation Curie-Institut du Radium, Section de Biologie, 26 Rue d'Ulm, 75231 Paris Cedex 5, France.

[5] We thank Mrs. M. J. Plessis and Mrs. J. Samouel for their excellent technical assistance.

[6] The same decrease of 30% in tumor incidence was observed after initiation by another hydrocarbon, 7-methylbenzo[*c*]acridine, under the same conditions. No such decrease was obtained when a tensio-active product (Span 85), devoid of promoting action, was used instead of caffeine as an eluting agent. (Its dose was chosen to give the same speed of elution as that of caffeine for tritiated DMBA.)

TABLE 1.—*Spectral distribution of the energy in the radiation of the Ellipiol lamp (with reflector)*

Wavelength, nm	Microwatts, cm^{-2} at 1 m
578	94
546	103
436	61
405	35
365	58
334	2.4
313	41
303	20
297	10
293	1.6
289	3.9
280	6.0
275	2.0
270	3.1
265	12.0
258	1.7
254	20
248	4.0
240	1.1
Background 8%	10.1 (For wavelengths < 330 nm)
Total wavelengths < 330 nm	136.5

An Ellipiol mercury vapor lamp of medium pressure was used. The spectral distribution of energy in the rays of this lamp was accurately determined (table 1). The lamp was placed 1 m from the animals. The mice were exposed to the lamp five times/week for a total of 133 exposures in 27 weeks. Taking into account that all radiation was of wavelengths less than 330 nm, the daily dose was 7.3×10^4 ergs/mm^2 or a total dose of 1×10^7 ergs/mm^2.

That total dose regularly produced tumors on both ears in 85–90% of the animals. Half that dose (0.5×10^7) gave tumors in only 10% of them.

Chemicals.—Caffeine, theophylline, and uridine were purchased from E. Merck (Darmstadt, Fed. Republic of Germany); reductone was prepared and checked for purity by Dr. E. Bisagni in our laboratory; chloroquine-base, which is soluble in acetone, was prepared for us by Rhône-Poulenc (Vitry-sur-Seine, France).

Chemical treatment of the ears and examination of the animals.—Before each irradiation, 40 μl of a 0.2% solution of caffeine or theophylline in acetone/chloroform was applied to the right ears. The same amount of the solvent was applied to the left ears as a control. The solvent evaporated rapidly and had always disappeared when the irradiation began. A 0.2% solution of reductone in acetone, a 0.1% chloroquine base solution in acetone, and a 0.1% solution of uridine in acetone–alcohol were applied under the same conditions.

The ears were painted before and not after irradiation because the irradiation time was long (90 min), and because the skin, which has just received a strong dose of UV rays, reacted badly to organic solvents.

Twenty nonirradiated controls received the same 133 applications of caffeine without developing any epidermal lesions by the end of 700 days.

The mice were examined every 10 days. For each animal, the appearance and evolution of any neoplastic lesion was recorded on an individual card. Every ear free from obvious tumor at the end of the experiment was checked histologically.

Study of caffeine uptake.—Uniformly tritium-labeled caffeine, 33 Ci/mmole obtained from the Radiochemical Centre Ltd. (Amersham, England) was especially prepared for this assay in an acetone solution (5 mCi/ml). Forty microliters of that solution (about 200 μCi and 1.2 μg caffeine) was spread on the dorsal side of the right ear three times at 30-minute intervals. The skin was removed 10 minutes, 30 minutes, 1 hour, 6 hours, and 24 hours after spreading. Frozen sections of nonfixed tissue were prepared in a cryostat, spread on gelatin-coated slides, and dried in the cold. In this fashion, all the caffeine taken up by the tissue and dissolved in the cellular water and fats remained in situ. The radioautographic emulsions (Ilford G5) were protected against nonspecific chemical effects from the tissue by a vacuum-deposited layer of carbon. Exposure time was 5 days. Other ears were washed in saline, fixed in methanol–acetic acid, washed again, dehydrated and embedded in Epon, according to the usual procedure.

One-micron sections were prepared with a glass knife on the LKB microtome, spread on slides, and covered with Ilford K5 emulsion by the dipping method. After 6 months or more, the preparations were developed, fixed, and slightly stained with methylene blue.

Animals without prior irradiation and those that received a usual dose of UV radiation 1 day before caffeine painting were studied.

Cultures of cells.—C3H2K cells (Yoshikura) were cultivated in Eagle's minimum essential medium containing 7–10% fetal calf serum, 0.75% bicarbonate, and 0.30% glutamine; 5×10^4 cells in 2.5 ml medium were seeded/35-mm Falcon dish. They grew at 37° C in air with 5% CO$_2$.

For the epidermal cells, the dorsal skin of 4-week-old mice was depilated with wax and attached to an adhesive surgical tape. The epidermis was separated from the dermis with the keratotome of Castroviejo and digested in a 0.2% trypsin solution. After washing in a calcium–PBS solution, $1\text{–}2 \times 10^6$ epidermal cells were seeded in the 35-mm Falcon dishes in the medium described above and cultured in an atmosphere of 5% CO$_2$ in air.

Irradiation of cells in culture.—The cells were washed with PBS, drained as completely as possible, and then irradiated with a Philips germicidal lamp. The dose rate for wavelengths of 254 nm was 25 ergs mm^{-2} sec^{-1}, as measured with a Latarjet dosimeter.

Labeling.—The irradiated cells were immediately covered with the medium containing 10 μCi of [^{3}H]thymidine/ml, the specified activity of which was 5.0 Ci/mmole; it was obtained from the Commissariat Energie Atomique (Saclay, France). After 3 hours of incubation at 37° C, they were fixed and processed for radioautography.

Radioautography.—At the end of the experiment, the cultures were washed several times with PBS and fixed in Carnoy's solution. The bottoms of the dishes were cut free with scissors and each bottom was cut in half and fixed to a slide. The preparations were then treated with a solution containing 0.2% Nonidet P40 and 0.5% trichloroacetic acid. They were then washed for several hours with running water and

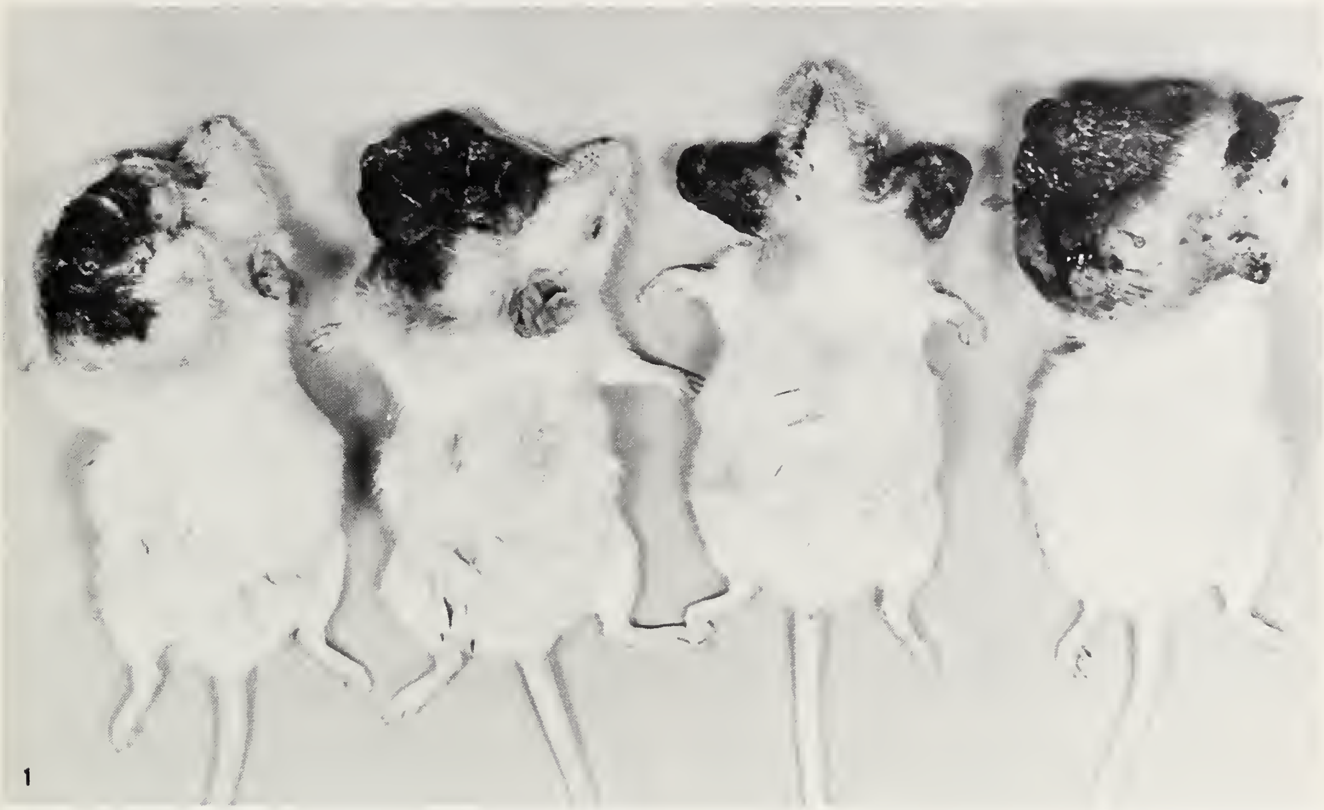

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FIGURE 1.—Four mice treated with caffeine on the right ear and killed in extremis. In 3, the right ear is free of tumor, whereas the tumor on the left ear has reached its final stage.

finally with distilled water. The preparations were covered with Ilford's K5 emulsion and developed in D19 after 28 days of exposure. They were stained with 0.5% azure A.

RESULTS

The cumulative dose of 10^7 ergs mm^{-2} of UV light delivered, as described above, to 600 mice, gave reproducible results. Tumors appeared on about 90% of the ears according to the same sequence of events. Following the classical inflammatory and congestive processes, the first tumors appeared 5 months and the last ones 11 months after the onset of the irradiation. Almost all these tumors were of epithelial origin; all were invasive and grew rapidly. After 5 to 6 months, they were two to four times larger than the head (fig. 1). The animals with malignant tumors on both ears were killed as soon as the macroscopic diagnosis was certain. Those with tumors on only one ear were killed 5 months or longer (when possible) after the appearance of the first tumor. Thus only few potential tumors escaped detection, primarily because of the strict hygienic conditions under which the mice were maintained.

Macroscopic and histologic examinations showed that most of the tumors were accompanied by cervical metastasis; 90% were keratogenic epitheliomas, and 10% sarcomas of subepidermal origin, recognizable by the absence of the kerato-hemorrhagic domes characteristic of epithelial tumors. Several tumors originated on the eyelids. At the end of the experiment, most mice were blind.

Inhibition of Carcinogenesis by Caffeine and Theophylline

These methylated oxypurines are generally considered capable of inhibiting the sealing of gaps in the DNA of UV-irradiated cells by the so-called postreplication repair. This inhibition is particularly conspicuous in rodent cells (*8, 9*).

Table 2 summarizes the results of these experiments from which the following conclusions can be drawn:

1) Tumor incidence in the left ears of animals that were used as controls was almost constant, varying from 84 to 89%.

2) In 2 experiments conducted independently, the incidence of tumors on the right ears that were treated with caffeine was only 54% in one and 47% in the other. The difference from the controls is highly significant ($P < 10^{-4}$).

3) Inhibition of tumorigenesis was the same (48% incidence) with theophylline.

4) The incidence of tumors (93%) did not diminish when uridine in alcohol and acetone was used to test the slim possibility that substances that strongly absorb UV rays could act through a screening effect. Uridine, a

TABLE 2.—*Incidence of tumors produced by UV on the ears: left ear, controls, UV alone; right ear, UV and substance*

Experiment No.	No. of animals	Tumors produced: Left ear No.	Left ear Percent	Right ear No.	Right ear Percent
1. Controls (no treatment)	56	48	86	53	94
2. Caffeine on right ear	55	49	89	30	54
3. Caffeine on right ear	57	48	84	27	47
4. Theophylline on right ear	54	46	85	26	48
5. Uridine on right ear	57	50	88	52	93

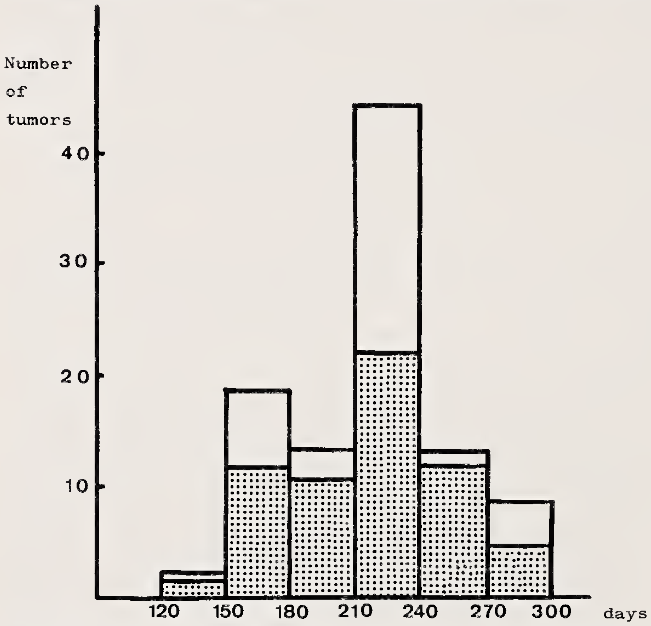
good absorber of UV, has no effect on the repair of DNA lesions.

5) The histograms of tumor latencies, similar in the controls and in the treated series (text. fig. 1), showed that the decrease in the frequency of the tumors on the treated ears is not due to delay in their appearance.

Assays With Reductone and Chloroquine

If the hypothesis is true that caffeine decreases the incidence of UV-induced cancers by inhibition of some error-prone postreplicative repair, then inhibitors of error-free prereplicative repair should have no effect comparable to that of caffeine. We tested reductone and chloroquine.

Reductone is the tartronic aldehyde $CH_2OH–CO–CHO$. Its mechanism of action is unknown. According to the work of Gomes et al. (*10*) and Leitã and co-workers (*11*), it is probable that reductone inhibits excision without inhibiting the postreplicative repairs in UV-irradiated *Escherichia coli.*



TEXT-FIGURE 1.—Histogram of the tumor latencies for the 2 experiments in which caffeine was used. Left ears, 97 tumors signified by *open bars;* right ears, 57 tumors signified by *dotted bars.*

TABLE 3.—*Incidence of tumors produced by UV on the ears: left ear, controls, UV alone; right ear, UV and substance*

Experiment No.	No. of animals	Tumors produced: Left ear No.	Left ear Percent	Right ear No.	Right ear Percent
1. Controls (no treatment)	56	48	86	53	94
2. Chloroquine on right ear	57	48	84	47	84
3. Reductone on right ear	55	49	88	50	89

It is unlikely that this compound could be presented as a "specific" inhibitor of excision. Its effects on yeast have not been studied.

In our laboratory, Yoshikura found reductone had no effect on the induction of endogenous C-type virus in mouse cells in vitro (unpublished observations). Zajdela studied its effect in vitro on UV-irradiated Yoshikura cells (from a C3H mouse), and the results he obtained so far are that important decreases are noted in cell survival (as happens in *E. coli*), in the incorporation of [^{3}H]thymidine in cells in S-phase (whether irradiated or not), and in unscheduled DNA synthesis in G_1-phase.

Chloroquine is an antimalarial agent derived from quinoline. We used a chloroquine base, which is soluble in acetone, whereas the chlorhydrate is water-soluble but insoluble in organic solvents. Contrary to quinacrine, it is not an intercalating agent. Yielding et al. (*12*) studied its action on the tumor response to X-rays and to alkylating agents. It inhibits protein synthesis, the transformation by Moloney and Rauscher viruses, excision repair in *E. coli,* and the repair of the DNA lesions produced by methyl methanesulfonate in rat liver cells in culture. At the same time, cell lethality is increased (*13*). We can consider that chloroquine acts principally on error-free modes of repair of UV damage (*14*).

We repeated the preceding experiments, using either reductone or chloroquine under the same conditions as with caffeine and theophylline, and found that the former drugs had no effect on the incidence of tumors (table 3).[7]

Radioautographic Analysis of the Penetration and Binding of Caffeine in the Epidermis

The radioautographs of frozen sections clearly show that caffeine in acetone penetrates the epidermis easily (fig. 2A). Caffeine is present at the surface, i.e., the cornified layer as well as in the cytoplasm and nuclei of the epidermal cells. It also readily penetrates the sebaceous glands, hair follicles, and the dermis. The precision of the cytologic localization is poor because of the thickness of the frozen sections (the sections of the nontreated control epidermis display a slight, uniform chemical fog).

The Epon-fixed sections from washed and fixed ears underwent an uncontrolled extraction by the alcohols and other histologic baths. One can surmise that the caffeine dissolved in water and in lipids was extracted. The radioautographs show radioactivity in all the nuclei of the germinal basal cells (fig. 2B), whereas none is in the layer of necrobiotic differentiated cells. Some residual activity can be found at the cornified acellular layer, probably due to incomplete extraction.

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[7] J. H. Epstein recently observed the same negative result with reductone (personal communication).

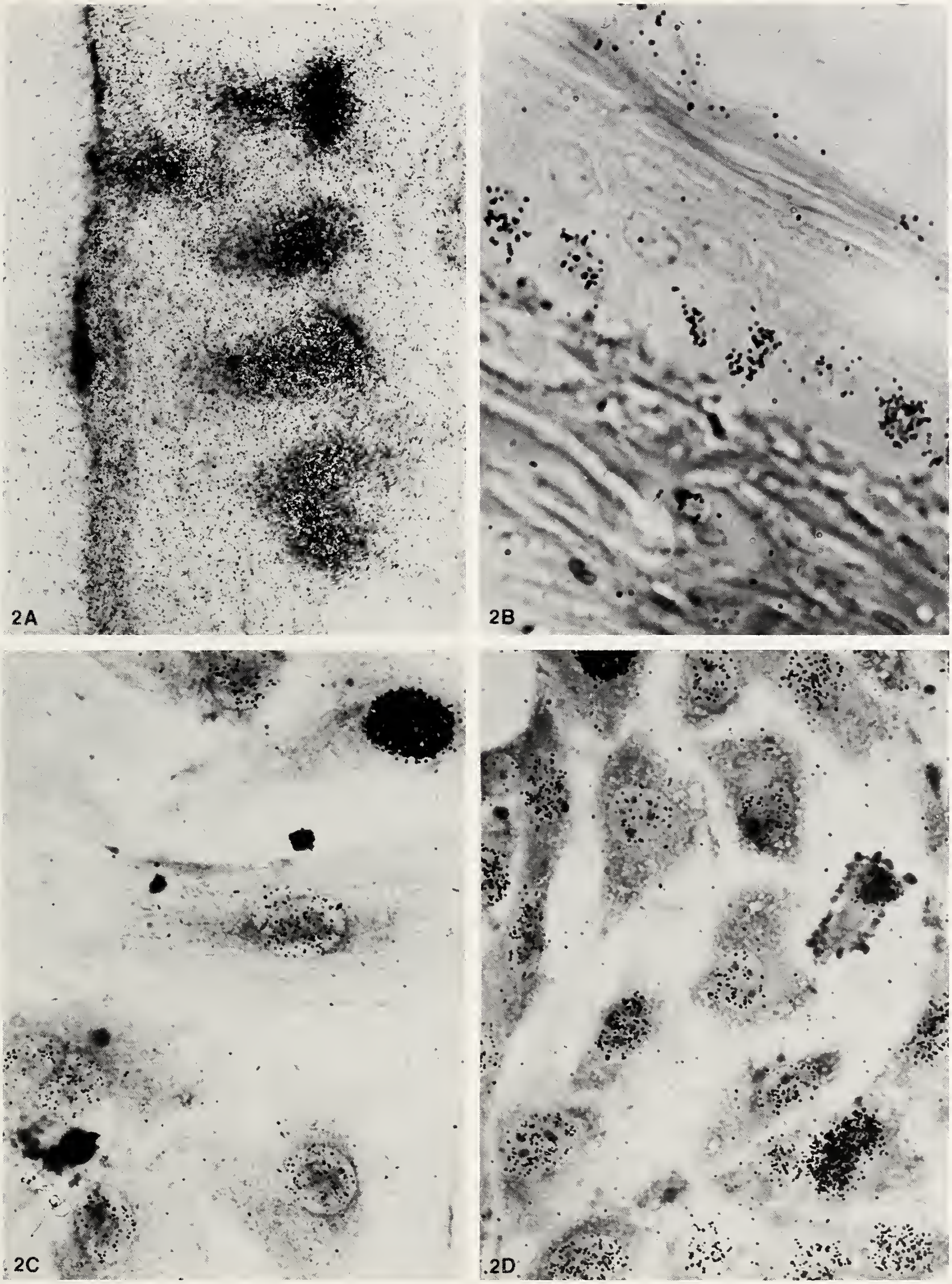


FIGURE 2.—A) Labeled caffeine on skin of the back, 10 min after painting (frozen section of nonfixed tissue; exposure, 5 days). Surface, hair follicles, and sebaceous glands are heavily labeled. B) Labeled caffeine in the skin of the ear, 30 min after painting (tissue fixed; caffeine dissolved in water and in fats has been extracted; inclusion in Epon; exposure time 7 mo). All nuclei of the basal layer are labeled; traces of label are also seen in the superficial keratin. C) Mouse epidermal cells in primary culture, labeled with [^{3}H]thymidine. Prereplicative DNA repair after UV irradiation (254 nm, 750 ergs mm^{-2}). D) [^{3}H]Thymidine in C3H2K cells 90 min after irradiation with 2,000 ergs mm^{-2}. On *center right,* one can notice a dying cell with a pyknotic nucleus which still incorporates thymidine.

It is difficult to determine the chemical nature of the radioactive compound present in the nuclei. That activity is weak, since an exposure time of at least 6 months was required for detection. It appears in the basal cells soon after the painting and is the same at 24 hours as it was at 30 minutes. Similar results have been observed by Rommelaere and Errera (*15*). Goth and Cleaver (*16*) have recently observed that caffeine is rapidly metabolized in several types of cells. After 1 to 3 hours, only 10% remains as caffeine (the methyl groups may be used in the synthesis of nucleic acid precursors).

Unscheduled DNA Synthesis in UV-Irradiated Mouse Cells and the Effect of Caffeine

The data on DNA repair in mice are contradictory (*17–22*). To clarify our situation, we identified DNA repair by radioautography after incorporation of [^{3}H]thymidine into G_1 and G_2 cells in culture, according to Stich and San (*23*).

Since in vivo conditions are not favorable for such a study, we used an established cell line of mouse origin, the C3H2K of Yoshikura (*24*), and primary cultures of epidermal cells of adult mice.[8] In these cells after irradiation, the distribution of the radioautographic grains denoting unscheduled DNA synthesis was regular, and the number of grains was proportional to the size of the nuclei. The radioactivity was expressed by the number of grains per 100 μ^2 of nuclear surface (average of counts about 100 nuclei). We did not try to curb the semiconservative normal synthesis of DNA with hydroxyurea or an arginine-deficient medium (*23*). In fact, the cells in S-phase could be easily recognized by their heavy labeling.

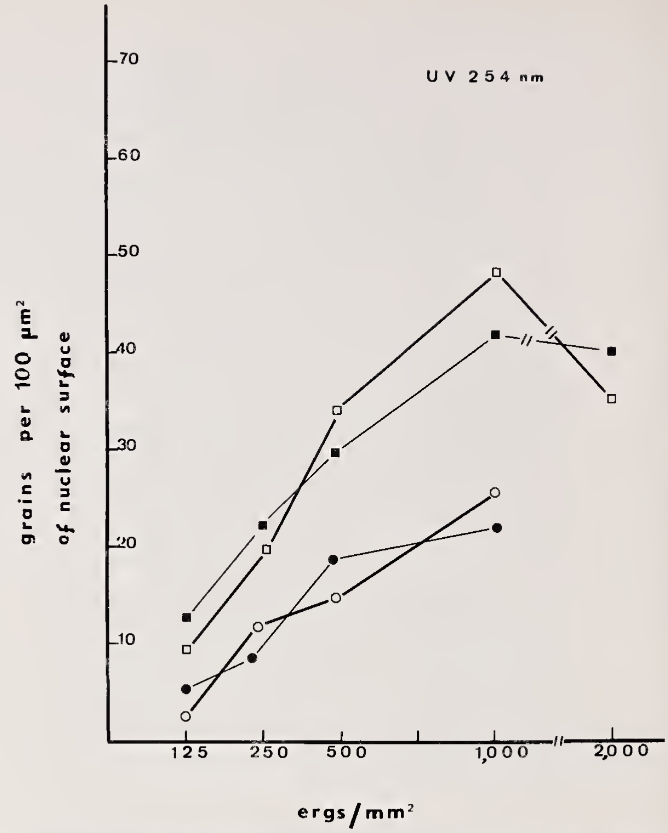
We used UV doses ranging from 40 to 5,000 ergs mm^{-2}. All the nuclei of C3H2K cells were able to repair (fig. 2D). The rate of unscheduled DNA synthesis increased with the dose of UV up to 1,500 ergs mm^{-2} (text-fig. 2). However, after a dose of 5,000 ergs mm^{-2}, some repair was still evident, despite signs of cell damage. After a dose of 1,000 ergs mm^{-2}, the repair decreased with time but could still be observed 20 hours after irradiation.

The primary cultures of epidermal cells of 4-week-old mice were also able to repair (text-fig. 2, fig. 2C). The rate of synthesis per unit of nuclear surface was about half that of the C3H2K cells, increasing at a slower rate with the dose.

The same experiment has been done in the presence of caffeine at a concentration of 0.7–1.5 mM, which is not cytotoxic. Caffeine did not influence this repair (prereplicative) in G_1 cells (text-fig. 2).

Only one experiment was performed with 1.5 mM theophylline after a UV dose of 1,000 ergs mm^{-2}. Here again, no effect was observed, but we must point out that our method is not sensitive enough to reveal differences of less than 20%.

These experiments show that the two mouse cell lines under study are able to repair UV-induced lesions in DNA. This conclusion agrees with those from other authors (*19, 20, 22*). The L-cells, which are unable to excise the pyrimidine dimers (*17, 18*), may be considered as peculiar. Many of them may have progressively lost their capacity for excision repair, as has been shown with other mouse cells by Peleg et al. (*25*).



TEXT-FIGURE 2.—No. of radiographic grains/100 μ^2 after [^{3}H]-thymidine incorporation in the nuclei of UV-irradiated cells in the presence and in the absence of caffeine, as a function of the dose of UV. □ = C3H2K cells; ■ = C3H2K cells + 1.5 mM caffeine; ○ = epidermal cells; ● = epidermal cells + 1.5 mM caffeine.

The radioautographic method does not define the nature of the observed repair. Since it was present in all the non-S-cells, it is probable that we were dealing with excision repair. Bowden et al. (*22*) reported that mouse epidermal cells can excise thymine dimers in vivo.

If, as is usually assumed, caffeine acts at the level of postreplicative repair, no effect should be detected by our method, a fact that agrees with the results of text-figure 2.

DISCUSSION

Carcinogenesis by UV rays is now considered as evolving from a primary photolesion in the genetic material of a cell. Since the doses of radiation necessary are enormous, it is difficult to realize how such a lesion-producing process, which expands as it progresses, leaves the cells alive, a condition necessary for the production of tumors. The process become clearer if we consider the capacity of the irradiated cells to repair their DNA.

It is generally recognized that prereplicative repair by excision is error free, at least after UV doses compatible with the operation of this constitutive repair system. The mechanism that leads to the excision of thymine dimers is a rate-limiting process, which is saturated at moderate doses of UV (*26*). It appears likely that the error-prone repair of DNA is postreplicative and inducible, at least in *E. coli* (*27*). UV light is one of the inducing agents of this type of repair by the action of the nonexcised pyrimidine dimers which stop the semiconservative DNA synthesis. Recent data suggest that this inducible "SOS" repair operates also in UV-irradiated mammalian cells (*28*).

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[8] Both spread nicely and are well suited for radioautographic analysis.

In our experiments, the doses of UV radiation required to elicit the malignant tumors of the epidermis exceeded those that allow the constitutive repair systems to remain operative. We had to use 1×10^7 ergs mm^{-2}, but the repair activity began to decrease at 1,500 ergs mm^{-2} (text-fig. 2). Our experiments with epidermal cells in vitro showed that, after high doses, affected cells were still capable of performing some unscheduled DNA synthesis in the presence or absence of caffeine.

However, alternative hypotheses can help to explain the inhibitory effect of caffeine on UV-induced skin carcinogenesis. The simplest one is that caffeine would increase mortality of cells capable of transformation and those already transformed through an increase in chromosomal aberrations (*8, 29*). The reductone, which is more cytotoxic than caffeine, does not modify the tumor yield. We will be unable to confirm this mechanism of action until we bridge the gaps in analysis between molecular, chromosomal, and cellular levels.

Presently, we cannot count the dead cells in the irradiated tissue because we lack a suitable methodology. In addition, the process involves an early hyperplastic reaction of the irradiated epithelium. To overcome this difficulty, we have undertaken some new analytical work.

Finally, one should take into account that caffeine and especially theophylline inhibit phosphodiesterase, which, by its lack of activity, produces an accumulation of cyclic AMP, and, consequently, a delay of cell growth. However, Ehmann et al. (*30*) recently concluded that "increased cyclic AMP levels do not now appear to be involved in the inhibitory effects of caffeine on DNA replication in damaged cells." We have undertaken a control experiment in which an analog of cyclic AMP, papaverine, and 3-isobutyl-1-methylxanthine, powerful inhibitors of phosphodiesterase (*31*), are applied locally (*32*).

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Pyrimidine Dimer Excision in Human Cells and Skin Cancer[1, 2]

James D. Regan, William L. Carrier, David P. Smith, Raymond Waters, *and* William H. Lee[3]

ABSTRACT—We compared three methods for estimating the induction and removal of UV-induced pyrimidine dimers from the DNA of human fibroblasts. Results indicate that after UV doses of 5–20 J/m², 50% of the dimers are removed by 24 hours. Almost complete excision can be observed if the cells are incubated for periods not less than 72 hours after a dose of 5 J/m². After higher doses, it probably takes even longer for such complete removal to be seen.—Natl Cancer Inst Monogr 50: 141–143, 1978.

Excision repair is a process by which cells repair damage to their DNA (*1*).

Patients with the genetic disease XP develop multiple actinic carcinomas on exposed regions of the skin (*2*). Such patients have either a defect in some step of the prereplication repair of UV-induced pyrimidine dimers, "classical" XP (*3, 4*), or a defect in postreplication repair, XP variants (*5*). These circumstances make it attractive to regard the unexcised pyrimidine dimer as the carcinogenic lesion in DNA. In postreplication repair, slow and perhaps inaccurate replication opposite a dimer has been suggested as a possible carcinogenic event. Thus to researchers of skin cancer caused by exposure to sunlight, the induction and fate of UV-induced pyrimidine dimers in the DNA of human cells is a subject of more than passing interest.

The purpose of this presentation is to describe our current results on the induction and excision repair of UV-induced pyrimidine dimers in the DNA of human cells. To obtain these estimates, we used three totally different approaches, two of which were pyrimidine dimer chromatography, which directly measures the percent of labeled thymidine existing as dimers in acid insoluble DNA (*6*), and a UV endonuclease incubation, which induces a single-strand break next to and in the same DNA strand as a pyrimidine dimer (*7*). These approaches directly estimate the number of pyrimidine dimers remaining in the DNA. The third technique, that of BrdUrd photolysis, estimates the number of pyrimidine dimers removed and repaired by excision resynthesis and rejoining and the average size of the repair region for each dimer removed (*8*).

The chromatographic assay involves the acid hydrolysis of the DNA to the free bases and acid-stable pyrimidine dimers and then separation by paper chromatography of the radioactive thymine from the thymine-containing dimers. Mammalian cells labeled with thymidine and irradiated with 20 J/m² of 254 nm UV light will result in approximately 0.1% of the radioactivity appearing as thymine-containing dimers. This corresponds to about 2×10^6 dimers/cell. Although dimers are formed between adjacent pyrimidines (thymine and cytosine) in the DNA, thymine-containing dimers are the most numerous and comprise about 85% of the total dimers (unpublished results). By using this method, we measured the induction and extent of removal of dimers from cells.

Table 1 presents data on the excision of UV-induced pyrimidine dimers in several human and other mammalian cell lines. We reported (*9*), as others have (*10–12*), that murine rodents differ significantly from man with regard to their excision capabilities. This fact must of course be taken into account in any studies of UV-induced cancers in experimental animals. For example (table 1), mouse 3T3 cells excise only 6% of their dimers in 24 hours, whereas one Chinese hamster cell line showed up to 50% excision in 24 hours. We found significant variations among different Chinese hamster cell lines ranging from 15 to 50% dimer excision in 24 hours. Human cells are capable of excising UV-induced pyrimidine dimers from their DNA. However, our previous experiments (*13*) indicated that this capability was limited, e.g., in the dose range of 5–15 J/m², only about 50% of the dimers were excised. Further studies by us and others confirmed this finding (*4, 14*). Since our earlier experiments, refinements in techniques of detecting dimers in small amounts enabled us to assay dimer excision at doses as low as 2.5 J/m². Under these conditions, human cells excise at least 95% of the pyrimidine dimers from their DNA. The human excision system appears to operate most efficiently at doses up to 30 J/m². The same absolute amount of dimers is not excised per unit time at higher doses. This explains how some workers fail to observe any appreciable dimer excision in cells receiving 100–300 J/m² (*15*). Thus normal human fibroblasts can excise essentially all their dimers at a dose of 5 J/m² of 254-nm radiation. However, to observe this complete excision, one must incubate cells for 72 hours after UV radiation.

Text-figure 1 is a graphic representation of the kinetics of UV-induced pyrimidine dimer excision in normal human fibroblasts. The solid circles indicate dimer induction and show the percent thymine as dimer that can be expected with these doses of 254-nm radiation. The other symbols represent the remaining dimers in the DNA at 6,

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Abbreviations: J = joule(s); XP = xeroderma pigmentosum; BrdUrd = 5-bromodeoxyuridine; M_W = weight average molecular weight; $1/M_W$ = reciprocal of M_W.

[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] Supported by Public Health Service contract Y01-CP50200 with the Division of Cancer Cause and Prevention, National Cancer Institute; contract T-1819E with the National Aeronautics and Space Administration, and a contract between the Department of Energy and Union Carbide Corporation.

[3] Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

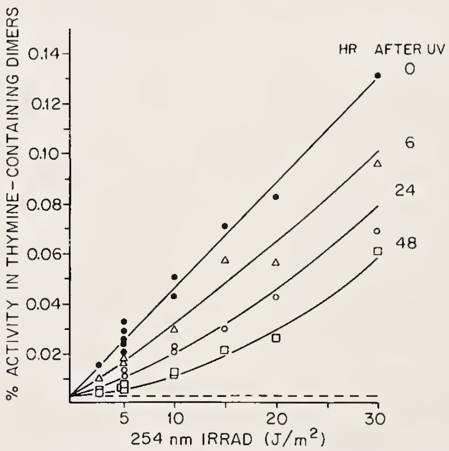
TABLE 1.—*UV-induced pyrimidine dimer excision in mammalian cells after approximately 5.0 J/m² of 254 nm radiation* [a]

Species	Cell type	Hr after UV	Percent dimers[b]	Percent dimer removal
Human	HSBP (normal fibroblasts)	0	0.025	0
		6	0.019	24
		24	0.010	60
		48	0.002	92
		72	0.001	96
	SGL-XP; (XP fibroblasts)	0	0.025	0
		24	0.024	4
		48	0.023	8
	Fanconi's anemia	0	0.028	0
		24	0.009	68
		48	0.005	82
Mouse	BALB/c 3T3	0	0.030	0
		24	0.028	6
Chinese hamster	CH23B	0	0.018	0
		24	0.009	50

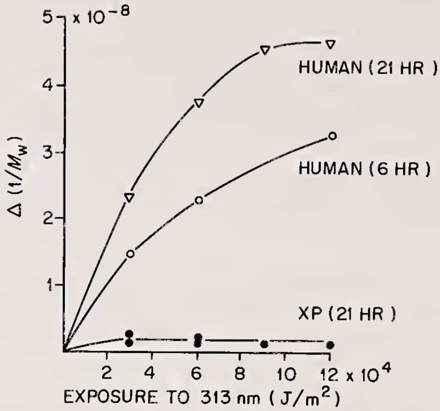
[a] Labeling of cells and irradiation were done as previously described in *(13)*. Dimer analysis was performed on the acid-soluble fraction of whole cells or isolated DNA according to Carrier and Setlow *(7)*.

[b] Average based on [³H]thymidine-containing dimers from at least 3 experiments. Radioactivity in the dimer region of the radiochromatogram of DNA from unirradiated cells was subtracted (0.001%).

24, or 48 hours after UV irradiation. Only at the lowest dose, i.e., 2.5 J/m² is complete excision achieved in 48 hours. Poon et al. (*16*) reported that fibroblasts from patients with Fanconi's aplastic anemia are defective in the excision of UV-induced pyrimidine dimers. We repeated these experiments with the Fanconi's cells used by Poon et al. (*16*) plus two fibroblast cultures from patients with Fanconi's anemia. We find no difference between Fanconi's cells and normal cells with regard to dimer excision (table 1). Thus the excision process does go to completion in human cells, but it is a protracted process. At UV doses of 10 or 20 J/m², which we and other investigators have used in the past, it is possible that 5 or 6 days would be required to excise 100% of the UV-induced pyrimidine dimers.



TEXT-FIGURE 1.—The amount of dimers remaining in the DNA of cultured human cells as a function of time after 254 nm of UV irradiation.



TEXT-FIGURE 2.—Relationship of the change in the $1/M_W$ to the dose of 313 nm radiation in human skin fibroblasts incubated in the presence of BrdUrd after 20 J/m² of 254 nm UV irradiation.

If dimer excision does proceed to completion, but slowly for 48–72 hours and longer depending on the dose of UV, then we would expect data from other repair assays to reflect the results obtained by pyrimidine dimer assays. If BrdUrd is supplied to the cell during the resynthesis step of excision, the cells will substitute the BrdUrd for thymidine in the gap filling process. The repaired DNA will therefore contain BrdUrd. The bromine of BrdUrd is labile (reactive) when exposed to 313 nm UV light. Sedimentation of the DNA in alkali results in a single-strand break occurring at sites of the photolyzed BrdUrd. The size of the DNA after 313-nm light treatment thus indicates the amount of repair taking place during incubation after UV irradiation. Text-figure 2 shows the results of experiments in which BrdUrd photolysis is used to measure excision repair. Normal cells after repair show a smaller DNA size, which indicates the presence of repaired regions (BrdUrd containing) in the DNA. Cultured XP cells demonstrate no change in the DNA size after incubation and 313-nm irradiation; hence few if any repaired regions are observed. For normal cells, the change in $1/M_W$ is not as great at 6 hours as at 21. This indicates that more excision repair took place between 6 and 21 hours, with only 50% of the dimer being removed at the later time.

Table 2 shows data from an assay of excision repair done by means of an endonuclease from *Micrococcus luteus*, which makes nicks in UV-irradiated DNA (*7*). Thus one can assay for the excision of dimers by quantitating the number of UV endonuclease-sensitive sites remaining in the DNA with time after irradiation. These data indicate that after the various doses of 254-nm radiation, about half the dimers remain unrepaired by 23 hours. Again, the Fanconi's cells excised dimers like the normal cells did. All three assays used therefore indicate about 50% of the dimers are removed by 24 hours after UV irradiation. This observation is contradictory to that of Amacher et al. (*17*), who found extremely rapid and extensive excision (70%) of dimers from human cells. We examined the excision of dimers in numerous other

TABLE 2.—*Endonuclease-sensitive sites*[a]

UV dose, J/m^2	Immediately after UV			23 hr after UV		
	Normal	XP	Fanconi's anemia	Normal	XP	Fanconi's anemia
5	13.5	15.2	13.0	5.1	13.6	4.6
10	27.8	29.9	28.1	12.0	30.0	11.7
20	55.0	56.5	55.8	24.5	54.1	27.1
30	81.0	83.2	—	42.8	81.7	—

[a] UV endonuclease-sensitive sites/10^8 daltons of total DNA in normal and XP fibroblasts either immediately or 23 hr after UV irradiation.

human cell lines and have not observed such a rapid removal. Calculations from the data available indicated that normal human cells excised the dimer equivalent to 0.5 J/m^2/hour.

Numbers of endonuclease sites were calculated as:

$$\frac{2}{M_w \text{ of DNA} + \text{UV endonuclease}} - \frac{2}{M_w \text{ of DNA} - \text{UV endonuclease}}$$

from computed M_w's (*16, 17*), as $M_n = \frac{M_w}{2}$ when M_w is random.

(M_w is random if one assumes pyrmidine dimers, and hence UV endonuclease-induced breaks are randomly distributed in the DNA. M_n = number average molecular weight.)

Can pyrimidine dimers in skin cell DNA cause skin cancer? We do not have the answer to that important question at present, but the XP data imply a correlation. Therefore, unequivocal information on the induction and fate of dimers is essential. To conclude, our past and present results indicate that dimers are removed by excision repair slowly and that repair must operate actively for 3–4 days to remove all the dimers by this mechanism. However, little data are available on how the process operates in different cell types and how culture conditions may affect it.

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Radiation-Enhanced Virus Reactivation in Mammalian Cells[1]

C. D. Lytle[2, 3]

ABSTRACT—Some doses of radiation to certain mammalian cells prior to their infection by UV-irradiated virus increased survival of the virus. This phenomenon has been demonstrated in several different irradiated (UV, X-ray, or photodynamic treatment) cell lines with UV-irradiated herpes simplex virus and with three types of UV-irradiated DNA viruses in monkey cells. Such radiation-enhanced reactivation of virus in mammalian cells may be analogous to the inducible, error-prone Weigle reactivation found in bacterial cell–virus systems. Inducibility of enhanced virus reactivation in mammalian cells is suggested by two types of results: 1) When UV-irradiated herpes virus infection is delayed 1 or more days after cell irradiation, higher levels of enhanced reactivation have been reported. Thus the reactivation increases with time. 2) Protein synthesis inhibition after cell irradiation prevents the expression of enhanced reactivation for subsequent herpes virus infection. Cycloheximide (10–30 μg/ml), a protein synthesis inhibitor, present in the tissue culture medium for 24 hours immediately following irradiation of monkey kidney cells prevented enhanced reactivation for UV-irradiated herpes simplex virus (infection immediately after the cycloheximide removal). This action suggested that radiation-enhanced reactivation in monkey kidney cells is inducible. In addition, enhanced reactivation was shown for UV-irradiated Kilham rat virus (which contains single-stranded DNA) in UV-irradiated rat nephroma cells, which provided evidence that UV irradiation of these cells led to overriding of UV-caused damage in the viral DNA. Together these data support the hypothesis that some aspects of radiation-enhanced virus reactivation in mammalian cells are similar to Weigle reactivation in bacteria.—Natl Cancer Inst Monogr 50: 145–149, 1978.

This paper will present a brief review and some new data concerning a phenomenon called "radiation-enhanced virus reactivation." Radiation-enhanced reactivation refers to the observation that the survival of UV-irradiated virus can be enhanced by irradiation of the host mammalian cell (*1*). Enhanced virus survival may be thought of as the assay for enhanced virus reactivation. Enhanced virus reactivation in mammalian cells is phenomenologically similar to Weigle reactivation in bacteria (*2*). However, since the mechanisms that produce Weigle reactivation in bacteria and enhanced reactivation in mammalian cells may be different, the terminology is probably best kept distinct.

Historically, enhanced reactivation was found during a series of studies to determine whether the different reactivation mechanisms for irradiated bacteriophage (*3*) might also occur in mammalian cells (*1, 4*). To date, several investigators have reported that host cell excision repair (*5–8*), postreplication repair (*9*), photoreactivation (*10, 11*), and radiation-enhanced reactivation (*1, 4*) can contribute to higher survival for UV-irradiated animal viruses in cultured mammalian cells. The importance of these different contributions depends on the situation: virus type, cell type, assay conditions, and treatment of the cell cultures before or after virus infection (*12*).

Radiation-enhanced reactivation usually occurs in only a few virus-infected cells (*13*) and normally contributes less to the survival of UV-irradiated herpes virus than excision repair or photoreactivation. The efficiencies (*3*) of these repair mechanisms in herpes simplex virus, as determined from dose reduction factors, can be as high as 0.6–0.7 for excision repair in human skin fibroblasts (*5*), 0.6–0.8 for photoreactivation in potoroo cells (*10*), and 0.4–0.6 for UV-enhanced reactivation in monkey kidney cells (*14*). Host cell postreplication repair ability is not important for survival of UV-irradiated herpes simplex virus, as found from similar survival in normal and XP variant cell cultures (unpublished observations). Day (*6, 9*) has clearly demonstrated, however, that cell-controlled postreplication repair, as well as excision repair, contributes to higher survival of human adenovirus 2.

Primary justification for further study of enhanced reactivation comes from the possibility that the responsible mechanism may be analogous to the inducible, error-prone Weigle reactivation of bacteria (*3, 15, 16*), which is highly mutagenic and is related to error-prone, mutagenic repair in bacteria (*17*). Increased mutability of mammalian viruses and cells by a radiation-induced scheme may be an important factor for radiation mutagenesis, carcinogenesis, and perhaps aging.

This paper reviews the state of knowledge regarding enhanced reactivation of UV-irradiated herpes simplex virus and reports some preliminary findings relevant to possible mechanisms.

Abbreviations: XP = xeroderma pigmentosum; SV = simian virus; SA = simian adenovirus; HCR = host cell reactivation; SOS = induced processes controlled by *recA* and *lexA* genes in *Escherichia coli.*

[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] Bureau of Radiological Health, Food and Drug Administration, Public Health Service, U.S. Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, Maryland 20857.

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REVIEW

Enhanced Virus Reactivation is a General Phenomenon

Radiation-enhanced reactivation has been demonstrated for several viruses, different cells, for various treatments of the cells, and at different times after cell treatment. Evidence for the occurrence of enhanced reactivation of UV-irradiated herpes virus is summarized in table 1. UV irradiation of the cell culture produces enhanced reactivation in established lines such as with HeLa, RMT, or CV-1 cells, whereas enhanced reactivation was only found in normal fibroblast cultures, if the assay by infection with UV-irradiated virus was delayed. X-Irradiation of RMT or monkey kidney cell monolayers can also enhance reactivation of UV-irradiated virus. Photodynamic treatment of the cells with proflavine and visible light caused a qualitatively different response, in that the normal human cells expressed enhanced reactivation for the UV-irradiated virus for immediate as well as for delayed infection.

To ascertain whether the enhanced reactivation was limited to herpes simplex virus, several other viruses (SV40, SA7, vaccinia, and polio) were investigated in monkey kidney cells. Of the viruses tested, only for the nuclear-replicating viruses (SV40 and SA7) was UV- or X-ray-enhanced reactivation found (*21*).

Mechanism Possibilities

Little is known about the mechanisms underlying enhanced reactivation in mammalian cells. Changes in survival curves of UV-irradiated herpes virus suggest the possibility of two processes. Survival curves for this virus usually consist of two components. Irradiation of the host cells can result in changes in the slope and the intercept of the second component (*13*). However, since the reasons for the two-component nature of the survival curves are unknown, changes resulting from cell irradiation cannot now be interpreted.

Furthermore, the efficiency of enhanced reactivation in monkey cells is greater for high UV fluences to the virus, i.e., in the second component of the survival curve (*13*). Virus growth and development have been reported to be delayed for UV-irradiated herpes virus (*24*). Perhaps enhanced reactivation only occurs for slowly growing virus. In addition, growth of unirradiated herpes virus is slowed in UV-irradiated monkey cells (*25*). Thus enhanced reactivation could be a matter of greater HCR in irradiated cells in which the irradiated virus proceeds through the replication cycle more slowly, allowing more time for repair. This possible mechanism for radiation-enhanced reactivation could be of great consequence with regard to a hypothetical model of induced, error-prone repair. Although no direct evidence supports greater normal HCR as a mechanism, there is only suggestive evidence to the contrary. XP cells XP12BE, which possess a low capacity for repair replication (excision repair), unscheduled DNA synthesis, and HCR, express a level of UV-enhanced reactivation similar to that of normal human skin fibroblasts (*18*). Thus excision repair probably does not play a major role in enhanced reactivation. The function of some other repair system such as postreplication repair is presently unknown.

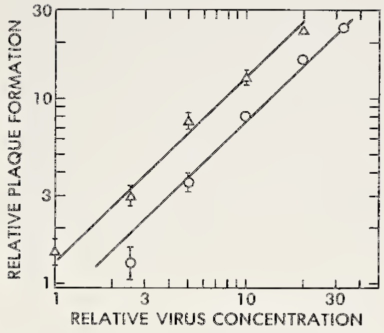
TABLE 1.—*Enhanced reactivation of UV-irradiated herpes simplex virus in mammalian cells*[a]

	Cell treatment						
	UV		X-Rays		Photodynamic		
Cell type	Immediate[b]	Delay[b]	Immediate	Delay	Immediate	Delay	References
Human							
KD	–	+[c]			+	+	*(18, 19)*
XP12BE(A)	–	+					*(18)*
XP11BE(B)	–	+					*(18)*
XP1BE(C)	–	+					*(18)*
XP7BE(D)	–	+					*(18)*
HEL	–						*(20)*
HeLa	+	+					*(20)*
HeLa (HVJ)	++	++					*(20)*
Monkey							
CV-1(TC-7)	++	++	++	++			*(21)*[a]
CV-1(ATCC)	+	++	+	+	++	++	*(14, 19)*[a]
MA134	+		+	±			[a]
Potoroo							
PtK2	–	+					*(22)*
Rat							
RMT	++		++				*(23)*
OMRE	–		–				*(23)*

[a] Data are author's (unpublished).

[b] Immediate refers to virus infection within 15 min of cell irradiation; delay refers to infection 24 or more hr after cell irradiation.

[c] – = reactivation factor less than 1.2; + = 1.5–3; ++ = greater than 3. Virus survivals were the order of 1%. Reactivation factor is the ratio of survival fraction of irradiated virus in irradiated cells to that in unirradiated cells *(20)*.



TEXT-FIGURE 1.—Relative plaque formation by UV-irradiated (465 J/m²) herpes simplex virus (MP strain) on UV-irradiated (9 J/m²) (△) and unirradiated (○) monolayers of CV-1 (TC-7) monkey kidney cells at different virus concentrations in the range used in enhanced reactivation experiments. *Solid lines* were drawn with a slope of 1.0. Since the number of plaques caused by UV-irradiated virus was proportional to the virus concentration, multiplicity reactivation did not contribute significantly to the survival on UV-irradiated or unirradiated monolayers. Techniques for cell and virus growth, plaque assay, UV irradiation, and dosimetry have been reported *(27)*.

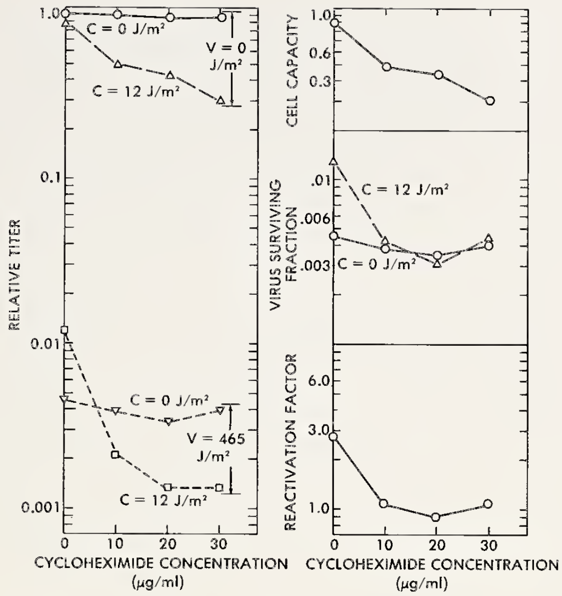
NEW INFORMATION

Multiplicity Reactivation

Multiplicity reactivation could represent another potential mechanism to explain enhanced survival in irradiated cells (*26*). Multiplicity reactivation can occur when there is more than one virus particle per cell, presumably by genetic recombination between the genomes of the different virus particles as a means of eliminating UV-caused damage in the participating genomes. Multiplicity reactivation can be demonstrated if the survival of irradiated virus is dependent on the concentration of the infecting virus. Although multiplicity reactivation cannot normally be demonstrated at the survival levels used for enhanced reactivation experiments with herpes simplex virus (*27–29*), it has been demonstrated with another herpes-type virus, human cytomegalovirus (*29*). Irradiation of the host cells could conceivably modify the virus–cell interactions to allow multiplicity reactivation. Text-figure 1 shows that the number of plaques caused by irradiated herpes simplex virus is proportional to the number of infecting virus particles when assayed in UV-irradiated and unirradiated monkey kidney cells. Thus for this virus–cell system, the titer (survival) of the irradiated virus remained constant at the different virus concentrations used in enhanced reactivation experiments. Therefore, multiplicity reactivation does not contribute significantly to UV-enhanced reactivation for herpes simplex virus in UV-irradiated monkey kidney cells.

Enhanced Reactivation May Be Induced

Because most of the repair phenomena found with bacteriophage can also be demonstrated with animal viruses, it follows that the process that provides Weigle reactivation in bacteria may provide radiation-enhanced reactivation for herpes virus in animal cells. Thus enhanced reactivation may also be inducible.



TEXT-FIGURE 2.—Effect of different concentrations of cycloheximide on UV-enhanced reactivation of UV-irradiated herpes simplex virus in CV-1 (TC-7) monkey kidney cells. Cell monolayers were UV-irradiated (0 or 12 J/m²), incubated for 24 hr at 37° C in minimum essential medium (with 10% fetal calf serum and twice the normal concentrations of amino acids and vitamins) containing different concentrations of filter-sterilized cycloheximide (Calbiochem), rinsed twice with Dulbecco's phosphate-buffered saline, and infected with virus. *Left panel* shows the titers for irradiated (465 J/m²) virus (▽, □) and unirradiated virus (○, △) and on irradiated (△, □) and unirradiated (○, ▽) cells relative to that of unirradiated virus on unirradiated cells with no cycloheximide. *Right panel: top,* capacity of UV-irradiated cells after incubation with different cycloheximide concentrations to support plaque formation by unirradiated herpes virus; *middle,* surviving fraction of irradiated herpes virus on UV-irradiated (△) and unirradiated cells (○) after incubation with different cycloheximide concentrations; *bottom,* reactivation factor after incubation with different cycloheximide concentrations.

In experiments with several cell lines (table 1), the amount of enhanced reactivation increases when the virus infection is delayed for some time after cell irradiation. For normal human and XP skin fibroblasts, enhanced reactivation is only expressed for delayed infection (*18*). The increase of enhanced reactivation with time after cell irradiation is consistent with, but does not prove, the hypothesis that enhanced reactivation is induced.

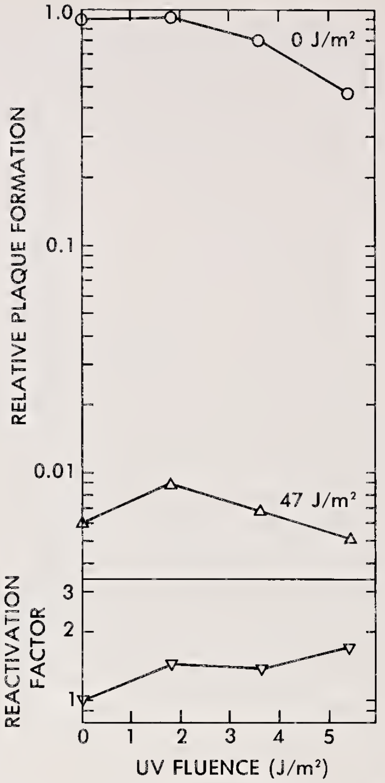
A more direct approach to investigate induction is by inhibition of protein synthesis during the time between cell irradiation and assay of enhanced reactivation by virus infection. This was done by the addition of 10–30 μg cycloheximide/ml to cultures immediately after irradiation, removal of the cycloheximide 24 hours later, and an assay of the enhanced reactivation by virus infection. Text-figure 2 shows the results when CV-1 monkey kidney cells and herpes simplex virus were used. With unirradiated cells, the cycloheximide treatment had little effect on plaque formation by UV-irradiated or unirradiated virus, whereas for the UV-irradiated cells, plaque formation by irradiated and unirradiated virus was decreased. Thus decreased capacity of irradiated cells to support virus plaque formation was lower when the cells were incubated for 24 hours in medium containing cycloheximide than for those without it. Without cycloheximide, the enhanced reactivation was at the expected level for this experimental system (*14*). However, the presence of cycloheximide (at all concentrations used) during incubation of the irradiated cells before virus infection inhibited enhanced reactivation as denoted by the reactivation factor being one (i.e., the same virus survival as in unirradiated cells). This result is preliminary in the sense that many corollary experiments are necessary to define more clearly the role of cycloheximide or other protein synthesis inhibitors in enhanced reactivation. However, the data strongly suggest that inhibition of protein synthesis after cell irradiation can inhibit subsequent expression of enhanced reactivation. A possible mechanism to explain these results would be the inhibition by cycloheximide of synthesis of a radiation-induced protein, the function of which could lead to enhanced reactivation of the irradiated virus.

Other evidence in irradiated mammalian cells also suggest an induction of enhanced repair. Setlow and Grist (*30, 31*) demonstrated that pre-irradiation of cells with UV or X-rays followed by an incubation period enhances the amount of postreplication repair after a subsequent dose of UV. D'Ambrosio and Setlow (*32*) found that the UV enhancement of this repair in Chinese hamster cells can be inhibited by incubation with 5 μg cycloheximide/ml, a protein synthesis inhibitor, during the time between the initial and subsequent UV doses. These findings parallel the situation found in bacteria when some postreplication repair, which is error prone, is induced by cell irradiation (*33*).

Evidence for Override Repair

Whether UV irradiation can induce an error-prone repair system in mammalian cells has not been confirmed. In searching for an inducible mutagenic system in mammalian cells, Cleaver (*34, 35*) found that X-irradiation of Chinese hamster ovary cells does not affect cell mutagenesis produced by subsequent UV irradiation for incubation periods between irradiation periods up to 17 hours. He concluded that X-rays do not induce a mutagenic repair system in these cells. The relationship of this finding to radiation-enhanced virus reactivation has not been established.

A series of experiments by Radman and co-workers (*16, 36*) showed that UV-irradiated *Escherichia coli* induced a repair process that can "override" UV damage. They demonstrated Weigle reactivation for the bacteriophage ϕX174 in irradiated *E. coli.* Since the DNA of this phage is single-stranded DNA, no dark repair, such as excision or recombination-type repair, takes place unless the first DNA synthesis of the replicative form (double-stranded) DNA can override the UV damage in the original infecting single-stranded DNA. This override repair process is induced by cell irradiation and appears with the same kinetics as SOS repair and mutagenic activities (*16*).



TEXT-FIGURE 3.—UV-enhanced reactivation of single-stranded DNA virus (Kilham rat virus) in cultured rat nephroma cells. Cells, irradiated as monolayers (5×10^5 cells/petri dish) at different UV fluences and incubated at 37° C with medium for 6 hr were infected with UV-irradiated (47 J/m^2) (△) and unirradiated (○) virus. Plaque formation relative to that for unirradiated virus on unirradiated cells is shown in *upper graph.* The reactivation factor (normalized ratio of *upper curves*) is shown in the *lower graph* (▽). Increase of the reactivation factor indicates UV-enhanced reactivation.

Thus enhanced reactivation of a single-stranded DNA virus in mammalian cells would provide evidence that override repair can be induced in these cells. Kilham rat virus is an appropriate test virus, since it has single-stranded DNA and probably no dark repair in unirradiated cells (*37*).

Kilham rat virus had higher survival when the host cells have been UV-irradiated and incubated before infection (text-fig. 3). Thus some override repair can be produced in irradiated rat nephroma cells. The increased survival indicates that the efficiency of this repair is about 0.08. These data represent the beginning of a series of experiments to investigate induced repair in mammalian cells with the use of single-stranded viruses. We expect to examine the kinetics of induction (if present), to detect whether the override repair is actually mutagenic, what fraction of irradiated cells is induced and, perhaps, whether excision repair or postreplication repair can further enhance survival after the initial override DNA synthesis.

SUMMARY

The available information on radiation-enhanced reactivation of UV-irradiated virus in mammalian cells has been discussed. The results were related to different possible mechanisms. Multiplicity reactivation was unimportant for enhanced reactivation of UV-irradiated herpes simplex virus. Some evidence was presented that suggested enhanced reactivation is an induced process. Other preliminary evidence for DNA synthesis overriding UV damage was shown for a single-stranded DNA virus. Future experimental questions to be answered will be directed toward ascertaining whether the process is indeed induced and/or error prone and whether it may be related to such other radiation-caused effects in mammalian cells as mutations and activation of latent tumor virus.

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Summary: Repair Processes and Their Role in Carcinogenesis[1]

Betsy M. Sutherland[2]

DNA repair in prokaryotes has provided the background for testing and understanding DNA repair in mammalian cells and may provide a framework for testing the role of DNA repair failures in human carcinogenesis. Dr. Kendric Smith has indicated that repair in *Escherichia coli* comprises both largely error-free pathways, i.e., oligonucleotide excision repair (via the *polA* system), base excision repair, and photoreactivation, and error-prone paths such as *recA*-mediated oligonucleotide excision and SOS repair. We have addressed two major aspects of DNA repair and its relationship to carcinogenesis: First, can we identify a premutagenic lesion in bacterial cells? Can we gain any information on such critical lesions in mammalian cells? What is the relationship between production of such a premutagenic lesion and carcinogenesis? Second, having identified (or at least selected) such a chemical alteration, can we delineate the biochemical and biologic steps leading from chemistry to carcinogenesis?

Although a sizable body of biologic, biochemical, and biophysical evidence has indicated that cyclobutyl pyrimidine dimers are important as a mutagenic lesion, no direct evidence has been obtained to correlate mutagenic sites with potentially dimerizable sites. Since the sequence of the *lac I* gene has been determined, and a large number of nonsense mutations in the gene accumulated, Coulondre and Miller have correlated mutation frequencies at specific sites with the DNA base sequence. Coulondre reports that the frequency of mutation is much higher in pyr–pyr sites than in pur–pyr–pur, in agreement with the identification of a pyrimidine run (two or more) as a potentially mutable site and a pyrimidine dimer as the initial lesion. A tantalizing problem that remains for the biophysicist are the apparent "hot spots" of high mutation frequency in some pyr–pyr sites.

If pyrimidine dimers are an important lesion in prokaryotes, can we say the same for eukaryotes? Although such an extrapolation in the absence of proof would be unwarranted, evidence is rapidly accumulating that this may indeed be true. The specificity of the photoreactivating enzyme for pyrimidine dimers has been used in the bacterial system as a tool for the analysis of the role of dimers in mutation and killing. The potential usefulness of the human photoreactivating enzyme in such a test depends on a demonstration of its specificity for pyrimidine dimers. The inability of the human enzyme to photoreactivate the cyclobutyl rings joining psoralen derivatives to a pyrimidine in a monoadduct may indicate a high degree of specificity of the enzyme. The photoreactivation of the lesion leading to UV-induced ability of human fibroblasts to grow in soft agar indicates that dimers are a prime suspect for the critical lesion in these cells as well.

Why should pyrimidine dimers be detrimental to cells if there are multiple pathways for their repair? Regan and Carrier presented evidence that dimers escaping the surveillance of the repair systems may be the lesion leading to cell killing and perhaps to solar carcinogenesis as well. How are these lesions converted to biologic damage? In bacteria, error-prone repair systems are thought to increase survival with concomitant increases in mutation. A signal feature of this "SOS repair" is its induction after the UV insult. Lytle found that an inducible repair system in mammalian cells functions to increase survival of UV-irradiated virus. Whether this inducible system is also error prone and thus a reasonable analog of the bacterial error-prone inducible system is not yet known. In bacteria, caffeine is an effective inhibitor of error-prone repair. Zajdela and Latarjet have examined UV carcinogenesis in the mouse and the effect of repair inhibitors on tumor formation: Agents which interfere with bacterial error-prone repair also reduce tumor incidence. These experiments provide a tantalizing hint that in mammalian cells an error-prone (inducible?) repair system may be responsible for conversion of UV-induced dimers to UV-induced tumorigenic lesions.

We have summarized many of the successes of the DNA repair story; we must also be careful to realize the limitations of extrapolation from prokaryote to eukaryote, mouse to man. However, we must retain our belief in biology and comprehend both the melody of biologic unity and the counterpoint of its diversity.

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Discussion: Repair Processes and Their Role in Carcinogenesis[1]

K. C. Smith: A lot of facts have been presented, with an inadequate amount of time to explain them properly, both from the point of view of how the data were gathered and what the implications of the results are. During this discussion period, you can question the different speakers to clarify some of these points.

Let me first put the subject in focus by saying that we now know that cells not only have different repair systems, but within each of these systems, multiple repair pathways are found. This is surprising, in a sense, because they seem to be repairing the same kinds of damage. Thus we can ask the question: Why are there so many multiple pathways in a given repair system? We can also ask the question: What is there specifically about different base regions within a gene that makes them more easily mutable? Does it mean that damage within these regions is easier or more difficult to recognize? We have inducible repair and constitutive repair systems. Some of these are error free, and some are error prone. We need to know how all the different repair systems interact. How are the lesions partitioned among these different repair systems?

In general, the take-home lesson is that mutagenesis is clearly due to errors in the repair of damaged DNA. In addition, evidence is growing that carcinogenesis is also due to errors in the repair of DNA. So with that little introduction, please direct your questions to specific speakers for clarification of any of these points.

H. Blum: As I pointed out in my paper, the growth of cancer, or of any other tissue, depends on proliferation and addition of cells. The uncertainty involved in measuring simultaneously the rate of cell division and the number of cells must be taken into account, because we cannot say that we are dealing with a transformation of cells or change in the rates of cell division, unless we have other evidence. I think this is often forgotten, and it needs to be kept in mind in all interpretations.

This does not apply to many of the things that have been said, but when we talk about growth of tissue volume, we should take this into account. I think that sometimes it has been assumed that transformation occurred when it really cannot be proved.

W. Lijinsky: I would like to make this comment: Dr. Smith, you said, almost categorically, and it rather dismayed me, that carcinogenesis is related to error-prone repair. This is an agreeable hypothesis, but the main danger, I fear, is its being converted into a dogma. There is, as far as I know, no direct evidence to support it, and, in fact, the evidence seems to rely on the correlation between mutagenesis and carcinogenesis.

A lot of people believe that mutagenesis and carcinogenesis are correlated, and that this has been demonstrated. I do not believe it. I think the evidence is poor. Even in Bruce Ames' figures, the correlation is 90%. Dr. Meselson has produced such a correlation, which Dr. Radman displayed in his slide. When I first saw that correlation on a slide last summer at a meeting in Cold Spring Harbor, it showed nitrosamines, and they were sort of glossed over, because they did not lie on the curve. In fact, they lay off the curve; so did the hydrazines. Now I see Dr. Radman's slide omits those two major groups of carcinogens entirely. This is an example of poor scientific data.

In some of my experiments, I took 43 polycyclic hydrocarbons and nitrosamines, and I or members of my group ran them in the salmonella mutagenesis test. Now for those (and I did select them carefully), I had quantitative carcinogenecity data in my laboratory, and 18 of 43 did not correlate. That means I had only a 60% correlation.

I think we have to bear in mind that bacteria and bacterial systems are not good models for carcinogenesis in metazoans, and I think we have to interpret the data most carefully. I agree that a bacteria mutagenesis test is a nice test to use, but we must remember that it does not represent carcinogenesis.

Smith: I should say that I was purposely a little bit dogmatic to help stimulate the discussion! I realize that this whole question of the molecular basis of carcinogenesis is currently fraught with emotion, but I challenge you to maintain an open mind as the new data on DNA repair, mutagenesis, and carcinogenesis are accumulated.

W. Clark: I would like to stimulate Dr. Regan, if I may, to get back to the issue that he eliminated at the early part of his paper. Then I think that, without stating that he eliminated it, he did not discuss the relationship between DNA repair and skin cancer.

In the first place, XP does not have a great deal to do with the development of skin cancer in man. It is a fascinating model, which may or may not be related to faulty DNA repair. A number of other defects of the skin occur in XP, including just overt holes, which never get an appropriate amount of melanin content and, throughout the life of the affected individual, let through an incredible amount of UV light.

Secondly, there are types of skin, e.g., such as mine, which have similar holes not related to any known or studied defects in the repair of DNA.

Now I would like to ask certain specific questions. First, in those patients who are prone to develop skin cancer along the axis of solar cutaneous atrophy, such as squamous cell carcinoma, of which specific phenotypes can be delineated, is there a defect in DNA repair?

Abbreviations: XP = xeroderma pigmentosum; UV-A = 320–400 nm of UV radiation; UV-B = 280–320 nm of UV radiation; TLCK = *N*-α-tosyl-L-lysylchloromethane; SOS (repair) = inducible cellular mutagenic and repair capacity.

[1] Conducted at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

Second, do those relatively fair (not dark) patients (e.g., the non-Celtic patients), who are not prone to skin cancer, have a difference in their DNA repair? In other words, have all these really elegant studies that have been done been related to cutaneous carcinogenesis in man?

J. Regan: Well, I doubt if I can answer all your questions. No one has done detailed studies of DNA repair in Celtic people versus more regularly pigmented people. Classical XP occurs among people with varying amounts of pigmentation and ethnic backgrounds. When you find a classical patient with XP, whether he be Irish, Jewish, or of other ethnic origin, there is a defect in DNA repair.

I did not mean to imply that we have positive, irrefutable evidence that pyrimidine dimers cause skin cancer. At the present time, however, an incredibly regular association occurs among the inability to remove dimers, the accumulation of dimers, and the appearance of skin neoplasms (in some patients, literally hundreds of neoplastic and preneoplastic lesions).

Clark: In XP, the skin cancer problem may be one of pigment distribution and may not be related to repair at all. Albinos also are deficient in protective pigment and are highly susceptible to skin cancer, presumably because a fantastic quantity of light passes through the skin.

Regan: If you are saying that patients with XP get multiple skin cancers because of a lack of pigmentation, that is not true, because patients with XP have different ethnic backgrounds, and among them certainly are representatives of the more pigmented races. I doubt if you can find a correlation between minimal pigment and the development of XP.

Now it is true, I agree, that albinos have a great risk of active skin cancer, which it seems to me is strictly due to a lack of pigment and hence an increased UV dose.

R. Johnson: We know that caffeine can be antimutagenic or can increase the UV-induced mutation rate in bacterial cells, depending on the concentration. Dr. Latarjet, have you used different concentrations of caffeine and found, with a particular dose, an increase in the number of tumors?

R. Latarjet: No; we did not and for two reasons. One is that these experiments are already so tedious and long that it would require several more years to try other concentrations. It may be done, but we have other things to do. The second reason is that it is impossible to quantitate the amount of caffeine that has been in contact with these living epidermal cells, which are candidates for transformation. Even if we could do this, we could not detect a saturation point.

I. Willis: My question is directed to Dr. Sutherland. One of your slides showed that photoreactivation of UV damage in *Escherichia coli* occurs at 360 nm, and within human cells, it occurs at 400 nm. Did you find that photoreactivation was wavelength-specific at 400 nm in human cells, or is this just the wavelength of *maximum* photoreactivation? Does photoreactivation in damaged human cells occur to any significant extent following UV-A exposure? This question is raised because of clinical results from our studies as well as results of studies by Drs. van der Leun and Parish that show an enhancement of UV-B damage (in the form of sunburn) to human skin by additional exposure to UV-A; however, we have also found (when using monochromatic light) that the longer the UV-A wavelength, the less is its photoaugmentation potentiality. This type of information may have important implications in designing sunscreening agents for human use.

B. Sutherland: The action spectrum peaks at about 400 nm and extends from about 300 to at least 650 nm.

We have used two broad spectrum light sources. One was a white light source, which the photoreactivating enzyme can use efficiently to catalyze the disappearance of the dimers. The white light, however, has detrimental effects on cells, so we used a yellow bulb, which the human photoreactivating enzyme can still use for dimer monomerization, but it does not have detrimental effects on the cells.

R. Pienta: Dr. Sutherland, you observed the growth of some human cells in soft agar after irradiation with UV. This implies transformation. Could you identify the source of these cells? Also, how long did you have to wait before you placed them into agar? Were you able to re-isolate them from agar? Our experience with some chemical carcinogens showed that this phenomenon might be a transient one, and unless you can isolate colonies, you cannot say much about them.

Sutherland: I agree that, at the present, evidence that the cells are transformed is lacking. What I can say is that they do grow in the soft agar, which Pollack finds to be the best index of correlation with tumorigenicity.

Pienta: Were the cells skin fibroblasts?

Sutherland: They were human embryo skin and muscle fibroblasts. How long did we have to wait after the UV? The schedule goes something like this: We set up the cells on Monday, expose them to UV during the week, and allow them to grow until the next Monday. Then we put them into agar, let them grow for 2 weeks, and then count the clones. I should add that immediately after the cells are placed in agar, the plates are scored for clumps to avoid possible confusion later.

You are absolutely right that we cannot say anything about their tumorigenic properties until we check their ability to produce tumors.

Pienta: Can you give any information on the size of the colonies or the number of cell generations that have elapsed in agar? How many cells are necessary for them to be considered positive when you see a clump of cells?

Sutherland: I try to score colonies of at least 32 cells. Many are much larger than this, and the exact size in the 2 weeks seems to depend on such variables as how many cells I put into the agar, how moist the agar plates are kept, and other culture conditions.

J. Robbins: I think it would be useful for me to expand on the comments that Dr. Clark raised about the relationship between the DNA repair defects of XP and their possible relationship to carcinogenesis.

Several points should help us to recognize that there is a great likelihood that the DNA repair defects are causally related to the development of skin cancer in these patients.

First of all, black people do get XP; there is no question about that. Its occurrence is reported in the literature, and we have observed it clinically.

Secondly, the white spots that occur in XP skin as areas of hypopigmentation occur in black and white people. We assume that these white spots arise from mutations in the melanocyte system.

Thirdly, I would like to point out that one major feature of actinic damage is death of cells in the epidermis and perhaps also in the dermis. In experiments in vitro, XP cells (e.g., fibroblasts, lymphoblasts) are more sensitive than normal cells to the killing effects of UV light. At least this actinic damage that patients with XP develop at an earlier age than do normal people is due to the effect of UV, which, in turn, is most likely due to the defective DNA repair of XP.

I do agree that it must be determined whether we can transform XP cells in vitro into malignant lines. These studies are now beginning, and we are going to irradiate XP fibroblasts and inject them into nude mice. We believe that if one considers all the clinical aspects of XP including the histopathology, as well as the natural course of the disease, little doubt remains that the skin cancer is due to the DNA repair defects that are present in these patients' cells.

Clark: Do not some patients with XP have normal DNA repair and still develop skin cancer?

Robbins: I know of no patients with XP in the world, which includes specifically those reported from Japan, Europe, and the United States, who do not have some DNA repair defect. Usually, this DNA repair defect is in the prereplication, excisional repair system. We found the first XP "variant" in 1971 and, subsequently, five others have been reported. These XP variants have a normal rate of excision repair, but they have a defect in some other aspect, perhaps one of the forms of postreplication repair.

In addition, Dr. Sutherland has shown that photoreactivating enzyme activity may be defective in certain patients with XP, and Dr. Linn reported that apurinic site endonuclease may be defective in patients of certain XP complementation groups. So it is becoming clear that many different defects may occur even within a given strain. In summary then, every patient with XP (of those currently reported) has some claimed defect in DNA repair.

Blum: We have known for a good many years that the penetration of UV light into the skin is largely based on the thickness of the epidermis. Thus one cannot trust the eye to judge how much penetration of light there is, because we may or may not have pigment, and still not have much penetration.

Latarjet: I have a question for Dr. Radman. One of the attendees has raised the problem of the validity of the bacterial mutatests. According to the multiplicity of inducible functions in relation to the SOS phenomenon, and since one of these functions is lysogenic induction, I would like to ask your opinion about the validity of lysogenic induction as used by Devoret and collaborators as a mutatest, rather than bacterial mutations as a whole.

M. Radman: It is clear that UV radiation activates or induces several bacterial functions, including the capacity to mutate, production of provirus in lysogenic bacteria, and inhibition of cellular division, the latter leading to filamentous growth. In a way, all tests that correlate with radiation and chemical carcinogenesis are a kind of "pH paper" or indicator that measures various consequences of nonexcised DNA damage. The induction of lambda phage is convenient, because it detects each cell that has suffered the induced SOS syndrome. In the mutagenesis test, the mutation must occur at the specific site to be detected. So I guess the question was: Why correlate one indicator, i.e., mutagenesis, and not something else, which is also induced by the DNA damage?

I would like to respond briefly to Dr. Lijinsky. Personally, I would never have had the intellectual courage of Meselson and Russell to look for a quantitative correlation between bacterial mutagenesis and carcinogenesis, because in animals, what one would really need is a study in which, instead of exposure dose, we would know the quantity of unrepaired DNA lesions. Having this information for bacterial DNA and for animal cell DNA, we could then look for the correlations. Otherwise, all metabolic activities that either destroy the carcinogenic chemical or eliminate DNA damage by prereplicative excision repair are obscuring a possible quantitative relationship. Ames thinks that nitrosamines are destroyed in salmonella, leading to an underestimated mutagenicity.

Many instances occur when we do not expect to get mutagenesis nor could we uncover it. For example, ethionine is a carcinogenic chemical that was not shown to cause DNA lesions, hence it should not be mutagenic and carcinogenic. Recently, it was reported that ethionine depletes the level of 5-adenosylmethionine because it competes for the charging, leading to 5-adenosylethionine. Therefore, the newly synthesized DNA is not methylated. From bacterial genetics, we know that the generalized methylation of DNA is important in the correction of errors, therefore, the under-methylation of DNA is mutagenic (Radman M, Wagner RE, Meselson M: Unpublished observations). I mention this just to illustrate that an apparent lack of correlation must not always be taken as proof of the lack of such a correlation. There are cases of mutagen-induced epigenetic phenomena in *E. coli,* but I will mention only the case of the lambda repressor. The lambda repressor is inactivated in lysogenic cells treated with carcinogens, e.g., UV radiation, and is destroyed by intracellular inducer(s), which appear(s) to be (a) protease(s). Lambda repressor is a self-controlling protein: When its level drops, the repressor stimulates its own transcription and translation, and when the level is too high, it represses its own transcription. If, as a result of this positive and negative control of lambda repressor synthesis by a lambda repressor, you destroy all of the repressor in *E. coli* by inducer and then eliminate the inducer, there will be no synthesis of the repressor, as if a mutation has occurred. Yet one can reactivate repressor synthesis if some repressor is provided by another lambda phage. This is an epigenetic phenomenon, which you can induce by a mutagen. Do such things occur in mammalian cells? Experiments by Mintz and Illmense show that in teratomas, one can suppress the initial teratogenic change.

I would like to mention briefly the arguments that make me think that carcinogenesis by chemicals and radiation is a genetic mutational phenomenon. The first argument concerns the stability and heritability. Once you have a transformed cell clone, it is as stable as any other known mutation. The malignant character of chemically induced and radiation-induced cancers appears to be recessive. This is concluded from the experiments of cell hybridization performed by Harris and Klein and collaborators. When a tumor cell and a normal cell are fused, the hybrid appears to be normal, as if the malignant change was corrected. However, after consecutive cell divisions, the tumor cells start to segregate and reappear again, and these tumor cells are the same as the initial ones. The conclusion is that malignant change was suppressed in the hybrid cell only as long as a specific chromosome from the normal cell was there. Once that chromosome was lost in the course of segregation, the tumor reappeared. That again suggests that, although the malignant change is recessive, it most probably is due to mutation, and once you eliminate the normal chromosome supplying the normal function, the tumor character appears. If malignancy were an epigenetic change, such as that one mentioned for lambda repressor, a permanent curing could be expected and not just suppression. Furthermore, the tumor and tumor hybrid cells do not complement, i.e., they never give a normal cell phenotype, which suggests that however you make a transformed cell, it is affected in the same pathway. This conclusion must be related to the possibility of noncomplementation for unknown reasons. On the other hand, we have no problem in imagining how single mutations could lead to pleotropic effects. When a single mutation causes several phenotypes, which apparently do not appear to be related to a single pathway, we call it pleiotropic. A single enzymatic activity could account for pleiotropy. From both *E. coli* and from cell hybridization experiments, we know that trypsin-like proteases can activate genes or gene products, e.g., G_1-blocked nuclei can be activated by active nuclei present in the hybrid, and this effect is inhibited by protease inhibitors.

To return to the problem of bacterial mutagenesis or animal carcinogenesis, it is important that transformation in vivo and in vitro occurs in two stages: initiation and promotion. Initiation is the event produced directly by the mutagen, and promotion appears to be a phenomenon, the mechanism of which is completely unknown but which leads to so-called "expression" of the transformed phenotype. In man, the delay between exposure to carcinogen until the appearance of visible tumors takes 10 to 20 years. Promoters, such as Croton oil, are not visibly carcinogenic themselves, but they increase the frequency of observed tumors, or of observed transformation in cell culture and make them appear sooner, as we heard from Dr. Mondal. I would presume that the simple way to think about it is that, in diploid cells, we have two copies of chromosomes. If we make a mutation in only one gene (and we have heard that the hybridization experiments suggest that transformation is recessive), the malignant change will be silent, i.e., suppressed by the presence of the other nonmutated chromosome. Consecutive cell divisions plus chromosomal rearrangements, mitotic cross-overs, or chromosome loss may finally lead to a mutational homozygosity, i.e., to a pure diploid mutant clone. I think this is an interesting problem to discuss. A measure of the extent of segregation in the production of mutant diploid cells is the comparison between the frequencies of dominant mutations appearing immediately in the diploid cells and recessive mutations.

L. Gorelic: Dr. Radman, you indicated that inhibitors of protein turnover had been used to ascertain whether protease activation was important in the induced repair phenomenon. I was wondering what levels of protein turnover in UV-irradiated cells were observed relative to the unirradiated control, and to what extent protein turnover was inhibited relative to an unirradiated control at the levels of TLCK used in these studies. I was also wondering if the protein turnover defective *deg*$^-$ *E. coli* mutants have been used in studies of induced (SOS) repair; if so, what were the results of such studies and how did they correlate with the currently acknowledged model for SOS repair?

Radman: The first question is related to the fact that TLCK, the synthetic protease inhibitor, is not a good substance to use because it is a weak alkylating agent and affects protein synthesis in general; it could act just like chloramphenicol. We have not measured the turnover of proteins. The concentrations of TLCK, which did inhibit UV-induced mutagenesis and prophage lambda induction, did not impair either viability or capacity to produce virus.

The second question was on *deg*$^-$ mutants. They are mutants that do not degrade inactive proteins or fragments of proteins, which are produced by nonsense mutants in *E. coli,* and all I know about this is that following UV irradiation, *lon*$^-$ or *deg*$^-$ mutants grow in filaments, and this growth is not reversible as in wild-type *E. coli.* This suggests the need for one protease to induce the SOS syndrome, including the filamentous growth, and for a second protease, *lon* coded or *lon* controlled, which would be needed to destroy the SOS-induced inhibitor of the cell division and to recover the cell division. In *lon* mutants, the second step is missing; therefore, the cells die after UV irradiation, i.e., they remain in filamentous form and do not form observable colonies. That is all I know about it.

Gorelic: My principle concern was whether the proteolytic enzymes associated with the enhanced level of protein turnover reported after UV irradiation of *E. coli* are, in fact, sensitive to the specific protease inhibitor used in your study. We have good evidence that, under starvation conditions, the protein components of *E. coli* can be classified into three groups on the basis of their turnover rates, and that the individual classes of cellular proteins differ substantially in response to the type of protease inhibitor used in your studies.

Regan: I would like to discuss with Dr. Radman his theory of tumor progression. Dr. Radman, you were saying we should imagine a diploid cell in which a spontaneous mutation is occurring at some locus on one chromosome. Somehow this cell would become homozygous for this mutation by a series of abnormal cell divisions, chromosome rearrangements, and so on. If that is true, then these recessive mutations must not be truly recessive, but rather leaky dominants, or else why should a cell that carries a recessive mutation undergo abnormal divisions, chromosome rearrangements, and so on?

The second mechanism you mentioned was the possibility of the cell becoming homozygous through spontaneous mutation at the opposite homologous locus. What do you think the probability is of two spontaneous mutations of the same kind, at the same locus, and occurring in the same cell?

Radman: Low, but that was not my statement. I guess the best way to test for any functional hybridization would be to take both high- and low-passage numbered cells and test them for mutagenicity of both recessive and dominant mutable loci.

The way I would approach this problem is to go to the literature and look for the frequencies of dominant mutations, where "homozygotization" is not required for the detection of the mutant character and compare them with the frequencies of all known recessive mutations. Dominant mutations are scored 20 to 50 times more frequently than the recessive mutations. This is not consistent with the recessive mutations and necessitates the coincident mutagenic event in both copies of the gene in a diploid genome. The latter would be rare enough to be practically undetectable.

The second type of experiment was done by Morgan Harris, who took diploid, tetraploid, and hexaploid cells and looked for frequency of recessive mutation. Now doubling or tripling the number of chromosomes in the case of recessive mutations should decline or just wipe out mutagenesis. That was not found. Again the factor of 20 appears. I have a specific model in mind, which I did not describe, that would require some blackboard work and that we are now testing in my laboratory. The question being tested is whether homozygous states could be brought about simply by crossovers between the centromere and mutation, followed by further division, i.e., segregation.

Smith: I noticed earlier that some of the immunologists seemed to be unhappy about the DNA repair model of carcinogenesis, but I would remind them that the immunologic surveillance systems would have nothing to act on if there had not first been a transformed cell, which seems to be produced by alterations in cellular DNA. If DNA repair were absolutely perfect, no cell changes would occur, and, therefore, the immune system would have nothing to work on! Does that stimulate any further questions?

S. Mondal: I have two technical questions for Dr. Sutherland. What was the percent of agar on your base layer and on the top layer?

Sutherland: The method is that of Ian McPherson.

Mondal: My second question is: What was the source of your UV light?

Sutherland: A 254-nm germicidal bulb. I should have added that we have action spectra on the schedule. Experiments for reciprocity are being done this week; had this Conference been a month later, I would have had some more data for you.

J. Epstein: Dr. Latarjet, we have done the experiment with the hairless mouse with topical reductone and with systemic chloroquine and obtained exactly the same findings that you did with topical chloroquine and topical reductone. We found no effect on cancer formation.

A second point is that, several years ago, we reported the presence of unscheduled DNA synthesis in the hairless mouse skin in vivo after UV irradiation. It was not nearly as much as we got in the human skin, but it was definitely there. So it confirms what you said.

J. DiPaolo: I concur that it is most comfortable to consider DNA mutations as responsible for transformation. However, although DNA is the critical site for a mutagen, the critical target(s) of chemical carcinogenesis is (are) unknown. The data that we have are also consistent with transformation being due to an epigenetic phenomenon. What you have been describing, when you talk about Klein and Harris in England, is a manifestation of the tumor properties of the cells, which is something else. Eventually, you have to get back to the nucleus.

Data obtained involving expression and suppression as a result of cell hybridizations have not been accompanied by thorough statistical analysis, nor have the protocols included studies involving the Luria-Delbrück fluctuation type of analysis. Harris and Klein hybridized transformed malignant lines and have reported that malignancy could be recessive or dominant.

Also, Barski has shown a difference in tumorgenicity between hybrid sister cloned lines that were karyologically similar, but only one of which produced a tumor.

I think that the evidence that demonstrates the relationship between transformation and mutation is not as strong as we would like to see it, particularly when we notice that if one scans the literature, the transformation data indicate a frequency of transformation many times higher than that obtained in mutagenesis experiments. I prefer to speak in generalities. I would have to say that the data as a whole at this point do not demonstrate a significant difference.

I am not sure if we are getting into the ridiculous. You have to have cell survival to get malignancy, so it is not a matter of just dose. If you do not have cells living, obviously you will have a sterilizing effect and you will fail to get cancer.

Smith: We could spend many hours discussing the right way to calculate and plot mutagenesis data, and it would be worthwhile, but that discussion is not a part of this session.

Johnson: My question is to Dr. Miller. Is there a difference in the pattern of hot spots between a cell that is excision deficient, as opposed to excision proficient?

J. Miller: We have not done those experiments to date on the excision-deficient cells; we are actually just starting them now.

R. Day: With *E. coli, Bacillus subtilis,* and *Micrococcus radiodurans,* it is clear that dimers end up inside the cell or outside the cell as trinucleotides. As far as I am aware, no data are available on this question for mammalian cells. Kuhnlein's paper[2] on the deficiency of apurinic site nuclease in XP group D cells made me think of the possibility that, during repair, a dimer-specific glycosidase is followed by some apurinic or apyrimidinic site enzyme. I thought at first that somebody must have data showing this idea to be wrong. Apparently nobody does. I did not consider it seriously and I still do not. However, since we have no data on this point, one cannot know that dimer excision in mammalian cells can be equated with dimer excision in *E. coli, B. subtilis,* and *M. radiodurans.*

Smith: What you are saying is that more work is needed, and that is certainly true in all fields.

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[2] Kuhnlein V, Penhoet EE, Linn S: An altered apurinic DNA endonuclease activity in group A and group D xeroderma pigmentosum fibroblasts. Proc Natl Acad Sci USA 73:1169–1173, 1976.

SESSION V

Host Influences on Ultraviolet Carcinogenesis

Chairman: P. Donald Forbes

Co-Chairman: Gio Gori

Influence of Heat, Wind, and Humidity on Ultraviolet Radiation Injury[1]

Donald W. Owens[2, 3] *and* John M. Knox[2]

ABSTRACT—We investigated the influence of heat, wind, and humidity on UVR-induced acute and chronic skin damage of experimental animals housed in environmental chambers and irradiated under controlled conditions. Hairless mice (strain HRS/J) irradiated after an increase of 10° F in skin temperature had more skin damage than irradiated controls. Significantly more Swiss albino mice irradiated for 400 days while maintained at 90° F developed tumors than did those receiving the same amount of UVR but maintained at room temperature. Mice exposed to UVR daily for 4 weeks while kept in wind of 7 mph had greater damage and slower recovery than animals irradiated but protected from wind. Wind also accelerated tumorigenesis in mice that received chronic UVR. Mice kept at 80% relative humidity and given a single dose of UVR had greater skin injury than animals irradiated while at 5% relative humidity. High humidity also appears to accelerate skin cancer formation in animals that were exposed to chronic UVR.—Natl Cancer Inst Monogr 50: 161–167, 1978.

INFLUENCE OF HEAT, WIND, AND HUMIDITY ON UV INJURY

Considerable effort has been devoted to the study of the adverse effects of UVR (*1, 2*), but environmental factors such as heat, wind, and humidity in UVR injury have largely been ignored. This report describes a series of experiments undertaken to assess the influence of these environmental factors in acute and chronic UVR injury (*3–5*).

Two environmental chambers, specifically designed for these experiments (fig. 1), provided thermostatically controlled temperature ranges extending from 40 to 120° F and a humidity index ranging from 5 to 100%. Additionally, wind tunnels built within the chambers provided a wind velocity of 7 mph (text-fig. 1). UVR was provided by fluorescent sunlamps (Westinghouse FS-40 T12; Westinghouse Lamp Division, Houston, Texas) installed within the chamber or by mercury arc lamps (GE Uviarc Model UA-3; General Electric Large Lamp Dept., Cleveland, Ohio).

TEMPERATURE

Acute UVR Injury

Materials and Methods

Three groups, each containing 6 hairless HRS/J mice, were treated as follows:

Group I.—This group was irradiated at room temperature (71° F) for 4 minutes (8 MED) with a mercury arc lamp. Skin temperature prior to irradiation was 85° F; immediately following exposure, it was 93° F.

Group II.—These animals were heated for 2 minutes with a hot water bottle (115° F) until the skin temperature rose from 85 to 98° F and then were irradiated with 4 minutes of UVR. Immediately after irradiation, the skin temperature was 95° F.

Group III.—This group was cooled for 2 minutes with a cold water bottle (32° F) until the skin temperature dropped from 85 to 58° F and then were given 4 minutes of UVR; their skin temperatures were 78° F immediately after irradiation.

Results

Group I.—Animals irradiated at room temperature developed distinct erythema and small scaling areas by the 3d day after irradiation.

Group II.—These animals developed more intense erythema, crusting, and necrosis of the skin by the 3d day after irradiation.

Group III.—Mice irradiated after cooling the skin developed mild erythema and small scaling areas by the 3d day after irradiation (fig. 2).

Chronic UVR Exposure and Carcinogenesis

Materials and Methods

Four groups, each containing 40 Swiss albino mice, were treated as follows:

Group I.—This group remained at 90° F and received 2.5 minutes of UVR (mercury arc) daily, 5 days/week for 57 weeks.

Group II.—The mice stayed at room temperature (75° F) and received 2.5 minutes of UVR daily 5 days/week for 57 weeks.

Group III.—These animals remained at 90° F and were not irradiated.

Group IV.—This group stayed at room temperature and was not irradiated.

All animals were observed for 400 days, and records were

Abbreviations: UVR = ultraviolet radiation; mph = miles per hour; MED = minimum erythemal dose; J = joules.

[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

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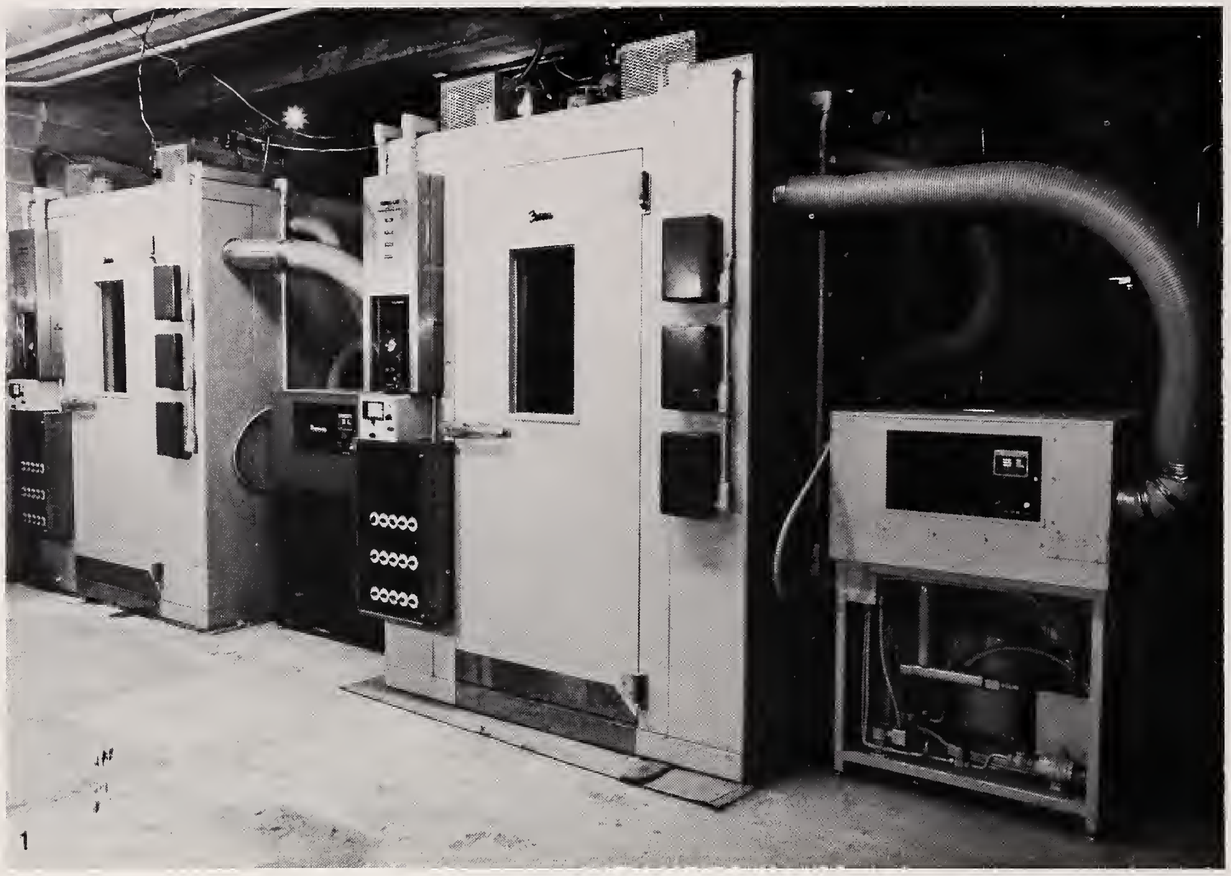


FIGURE 1.—Environmental chambers.

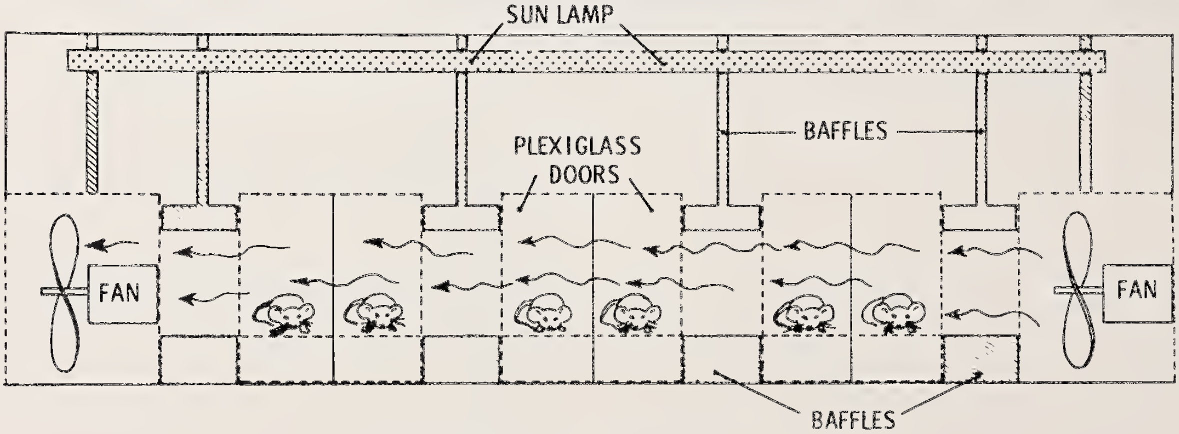
kept of the number of survivors and of the time the first tumor appeared in each mouse.

Results

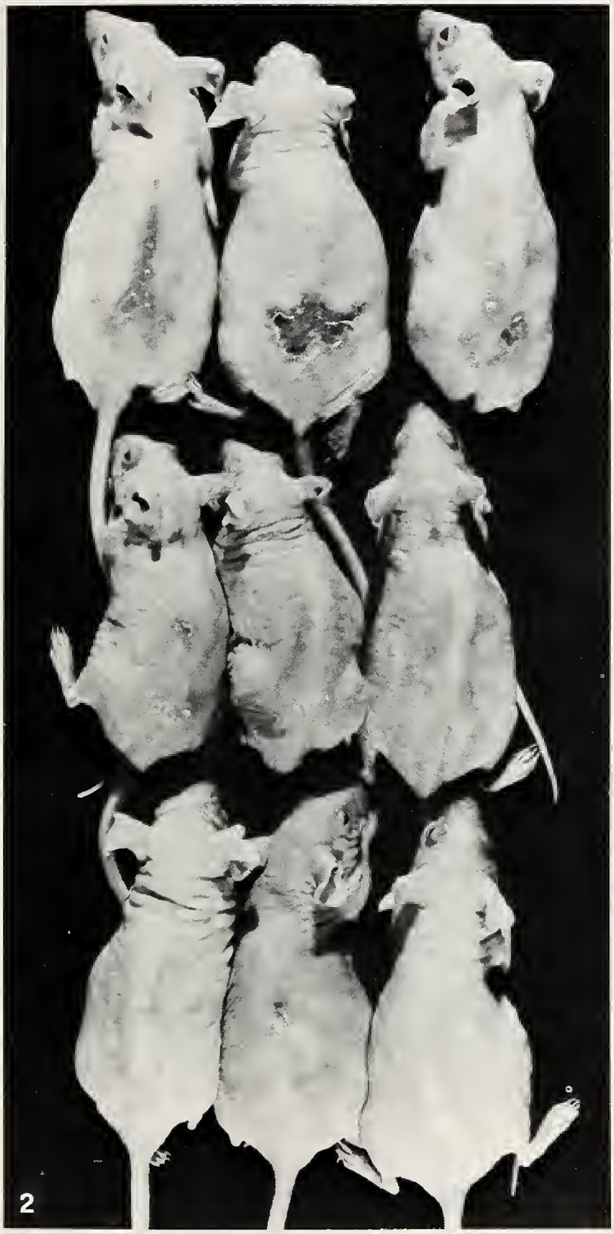
A constant high environmental temperature increased the number of animals that developed tumors and the rate at which these tumors developed (table 1).

All 21 irradiated mice that were maintained at 90° F (group I) and survived developed tumors within 260 days. The first tumor appeared after 180 days, and the average time of onset was 218 days. In mice maintained at room temperature and irradiated (group II), only 27 of 39 developed tumors within the 400-day observation period. The average time of onset was 285 days, even though the first tumor in this group appeared at about the same time as in group I. Only one of the controls developed a tumor. All tumors examined histologically were squamous cell carcinomas.

Only half the mice kept continuously at 90° F (groups I and III) survived 180 days. In all others, an average of 85% of the animals survived this length of time. Thus constant high



TEXT-FIGURE 1.—Interior design of chambers.



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environmental temperatures shortened the animals' life-spans.

Table 1.—*The effect of high environmental temperature on UVR carcinogenesis*

Treatment groups	Percent of survivors with tumors	Average time of onset, days
Continuous heat + UVR	100 (21/21)	218
Room temperature + UVR	69 (27/39)	285
Continuous heat only	5 (1/20)	206 (1 animal)
Room temperature only	0 (0/34)	

WIND

Acute UVR Injury

Materials and Methods

The chambers accommodated 3 groups of young adult, Swiss albino mice (18 mice/group): UVR and wind, UVR only, and wind alone. The wind velocity, measured with a vane-type instrument at six points in each cage, showed wind flow was constant and uniform throughout. Conditions in the chambers were maintained at 95% relative humidity and at 80° F. Animals were irradiated daily with 3 MED for 4 weeks by sunlamps (Westinghouse FS-40 T12) that were installed in the chambers.

Animals were taken from the environmental chambers daily and inspected for damage to the ears. Changes were recorded on a numerical scale ranging from 0 to 6 as follows: 0, no observable effect; 1, prominent veins; 2, slight erythema; 3, moderate erythema; 4, intense erythema; 5, scaling; and 6, sloughing of the ear margins.

Results

Animals exposed to UVR and wind had greater damage to their skin than animals exposed to the radiation alone (text-fig. 2). Damage to the animals' ears was similar in both groups until it reached a peak during the 2d week of exposure and then the animals gradually recovered despite continuous irradiation at the same dosage. This recovery was accompanied by gross thickening of the animals' ears. Mice receiving UVR and wind developed slightly more damage to their ears and had delayed recovery when compared with the animals receiving the radiation alone. The damage was statistically significant ($P < 0.05$) after the 12th day of the experiment. No detectable effect from the wind alone was observed.

Chronic UV Injury and Carcinogenesis

Materials and Methods

Young adult, female, hairless HRS/J mice were maintained in the environmental chamber under the conditions of 20% relative humidity at 90° F. One group of 30 mice received irradiation 5 days/week from 2 mercury arc lamps. The animals were removed from the chamber for irradiation and returned immediately afterward. A second group of 30 animals were irradiated identically but were also maintained in the wind tunnel within the chamber and exposed to a constant wind flow of 7 mph. The third group of 6 mice maintained in the chamber were exposed only to a constant wind flow of 7 mph.

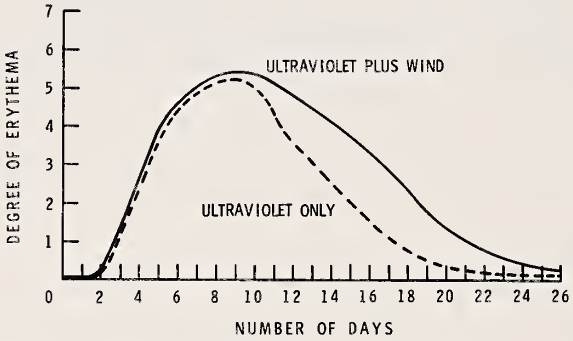
The irradiated animals received a suberythemal dose daily 5 days/week for 42 weeks (an increasing UV dose) to accommodate thickening of the skin from continuous exposure. The animals were examined weekly for development of skin lesions, and growth of a discrete tumor 2 mm in diameter was considered the end point. Total numbers of tumors or number of tumors/animal were not recorded. We excised tumors for histopathologic study in a random fashion in 20% of the animals.

Results

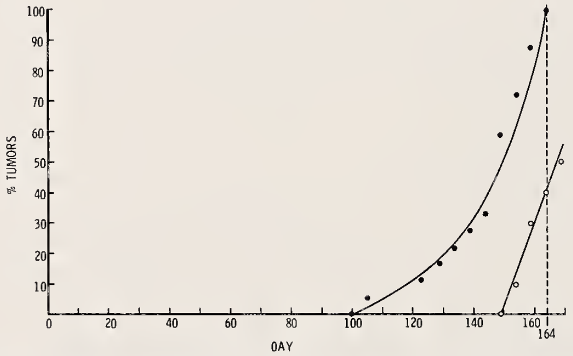
The first tumor appeared in the group treated with UVR and wind at 105 days of irradiation; by 164 days, all surviving mice developed at least 1 tumor (text-fig. 3). In contrast, the first tumor appeared in the group exposed to UVR but not to wind at 154 days of irradiation and by 164 days, only 40% of the mice developed tumors. The difference in the 2 groups was statistically significant from day 124 ($P < 0.05$). As the experiment progressed and more tumors developed, the significance increased to P less than 0.001 by day 164. The first tumors appeared in the groups exposed to UVR and wind and radiation alone at total cumulative doses of 3.80 and 6.45 J/cm^2, respectively.

All tumors examined histologically were squamous cell carcinomas. No animal exposed to wind alone developed tumors.

All the nonirradiated animals were alive at the conclusion of the experiment. Unfortunately, 12 animals in the irradiated, wind-exposed group died after 76 days of irradiation when the thermostat in the chamber with cooling failed to function properly 1 weekend. We observed death previously in animals cooled and exposed to wind. Since cooling of animals protected them from the effects of UVR, we believe the isolated event did not accelerate tumor formation among the surviving animals.



TEXT-FIGURE 2.—Damage from sunlamp with continuous wind exposure compared with that from sunlamp alone.



TEXT-FIGURE 3.—Prevalence (mice with tumors / No. of surviving mice). *Solid circles* = wind + UVR; *open circles* = UVR alone.

HUMIDITY

Acute UVR Injury

Materials and Methods

Environmental chamber studies.—Young adult, female, hairless HRS/J mice were housed under controlled conditions of temperature and humidity while they received UV irradiation from Westinghouse FS-40 T12 sunlamps. In all experiments, the chamber temperature was maintained at 95° F without wind flow.

Comparisons of UV damage were made among animals maintained at high, mid, and low humidities.

Experiment #1: Twelve hairless mice were maintained for 96 hours in the chamber at 80% relative humidity and were then irradiated with 10 MED from the sunlamp (1 MED= 275 mJ/cm^2). Following irradiation, the animals were maintained in the chamber under the same conditions for 72 hours and observed for damage. After the chamber was adjusted to 5% humidity, 12 hairless mice were kept for 96 hours and then irradiated with 10 MED.

Experiment #2: A similar experiment was performed, but a group of animals kept at mid-humidity (50%) was also studied.

Influence of hydration on MED determinations.—Male and female adult, albino rabbits were depilated (Surget depilatory, Crookes-Barnes Laboratories, Inc., Wayne, N.J.) 24 hours before irradiation with serially increasing amounts (from 2 to 20 mJ/cm^2 at 2-mJ/cm^2 increments) of UV rays at 300 nm emitted from a high-pressure, xenon arc grating monochromator. We treated the abdomens of 7 animals on one side with wet packs (tap water) for 4 hours prior to irradiation; the nonhydrated irradiated side served as the control. We examined the irradiated sites at 24 hours for erythema and then determined the MED.

Results

Environmental chamber studies.—By 72 hours, differences were maximal with the animals treated with high humidity showing prominent exfoliation, crusting, and erosion of the skin, and the groups exposed to low humidity having much less damage (fig. 3). The animals treated with mid-humidity had distinctly less scaling and crusting than the high-humidity groups but more than those exposed to low humidity. The only animals that died were from the high-humidity groups that received UVR. Seven of the 12 mice from the first experiment and 5 of 12 from the second expired within the week of irradiation, presumably because of acute UVR injury. The clinical observations were confirmed by histologic studies (fig. 4).

Differences were not seen among unirradiated control animals kept at high, mid-, and low humidities.

MED determinations.—In the MED studies with the monochromator, each of the 7 albino rabbits had lower energy requirements for erythema production on the hydrated side of their abdomens (table 2). The differences are statistically significant with P less than 0.01.

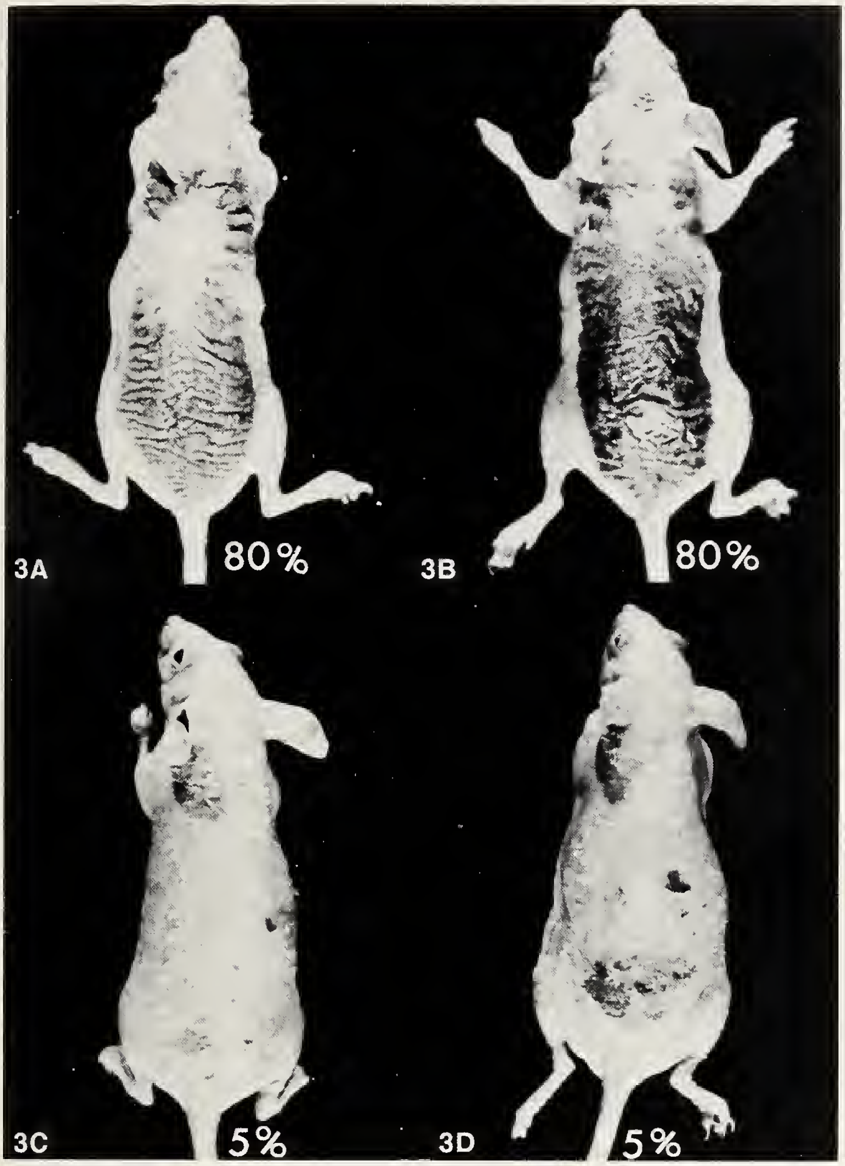


FIGURE 3.—Mice 72 hr after irradiation with 10 MED while maintained at 80% relative humidity *(top)*, and 5% humidity *(bottom)*.

Chronic UVR Exposure and Carcinogenesis

Materials and Methods

Young adult, female, hairless HRS/J mice were maintained in the environmental chambers at 90° F and irradiated 5 days/week from 2 mercury arc lamps. Two groups of 30 mice each were maintained at 20 and 80% relative humidity, respectively. The dosage of UVR was precisely the same as administered in the previously described study on chronic UV injury and carcinogenesis in mice exposed to wind.

TABLE 2.—*MED determinations in hydrated and dry rabbit skin*[a]

Animal No.	Hydrated, mJ/cm^2	Dry, mJ/cm^2
1	4	6
2	4	6
3	6	10
4	6	8
5	6	10
6	6	8
7	6	8

[a] Mean and SE for hydrated skin = 5.4 and 0.369; for dry skin = 8.0 and 0.61.

Results

Unfortunately, the study was terminated prematurely due to mechanical failure of the chambers and death of the mice maintained at high humidity after 95 days of irradiation. Nevertheless, the first tumor appeared in the high-humidity group after 76 days of irradiation, and when the experiment was terminated, 25% of the animals had tumors (text-fig. 4). In the mice that received identical irradiation but were housed at low humidity, the first tumor appeared after 154 days of irradiation, and by 159 days, only 30% of the animals had tumors. Although it is unfortunate that the study was not completed, the preliminary data show that high humidity accelerates carcinogenesis ($P < 0.05$).

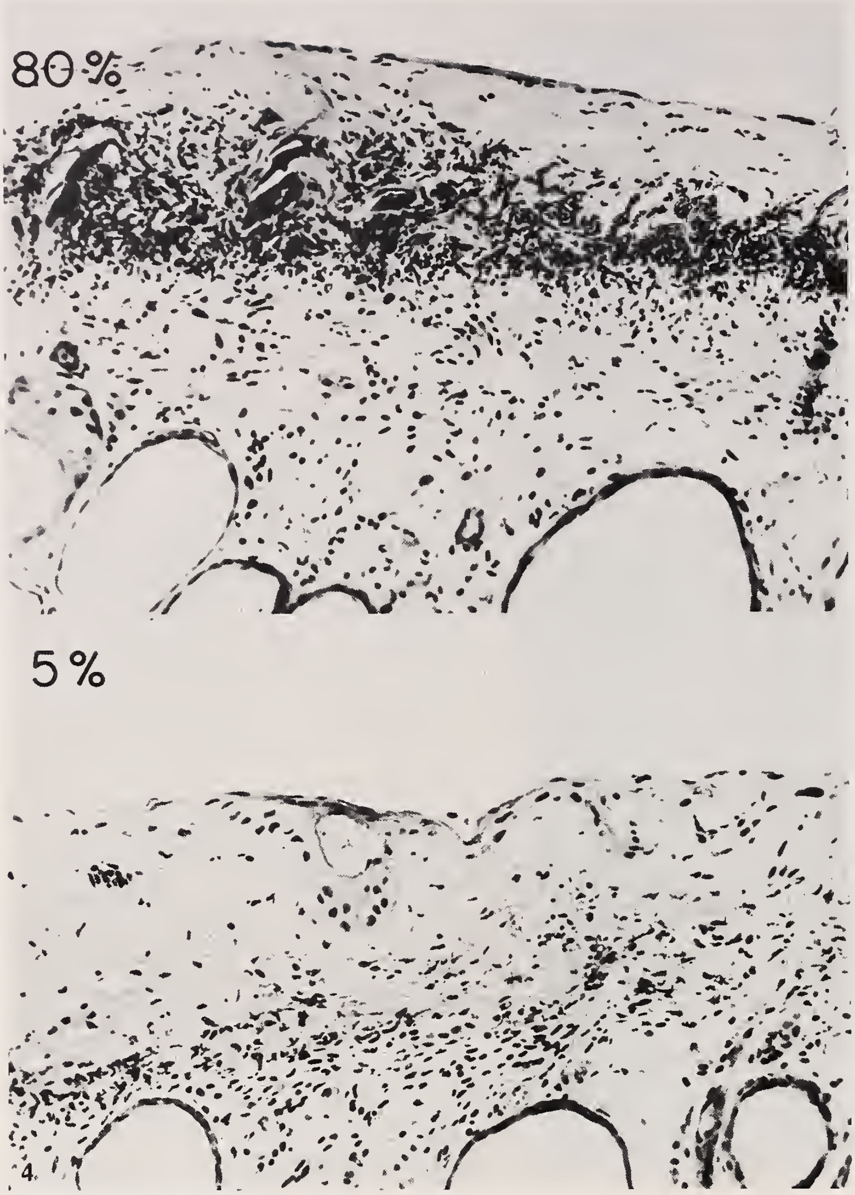


FIGURE 4.—Biopsy specimens from mice 72 hr after irradiation with 10 MED at 80% relative humidity and 5% humidity. × 160

DISCUSSION

The results of these experiments indicate that heat, wind, and humidity enhance acute as well as chronic UV injury and carcinogenesis; these influences should be considered in any biologic experiment on UV injury.

Skin temperature at the time of UVR influences the effect of that exposure. Mice with elevated skin temperatures during UVR developed more intense erythema and a greater amount of crusting than did those with normal skin temperature. Animals with lowered skin temperatures manifested less skin damage than those maintained at room temperature. The observation that mice living in a heated environment develop tumors more rapidly than those living in a temperate climate confirms the report of Bain et al. *(6)* that heat enhances carcinogenesis. These investigators exposed mice to a mercury lamp after subjecting them to temperatures of 40° F, room temperature, or 95° F.

Animals exposed to UVR and wind have greater skin damage than those exposed to UVR alone. Wind alone produced no observable effects. Although the damage was greater in the group exposed to irradiation and wind, the delayed recovery from this exposure was more impressive. Since wind adversely influences UVR-induced damage over a 4-week period, we were not surprised to learn that it also promotes tumor development. We believe that wind exposure did not lead to increased skin exposure to trace chemical carcinogens in the atmosphere because the chambers were housed in an airconditioned room and the air provided to the environmental chambers was filtered room air. No other experiments took place in the room where the environmental chambers are housed.

Increasing humidity and application of wet packs intensified acute UVR-induced skin damage as shown in the mice maintained at high humidity and irradiated when compared with the animals receiving the same amount of UVR but which were maintained at low humidity. Animals maintained at mid-humidity had skin damage intermediate between those kept at high and low humidity. Although the study was not completed, mice exposed to chronic UVR exposure at high humidity experienced more rapid onset of tumors than did animals maintained at low humidity.

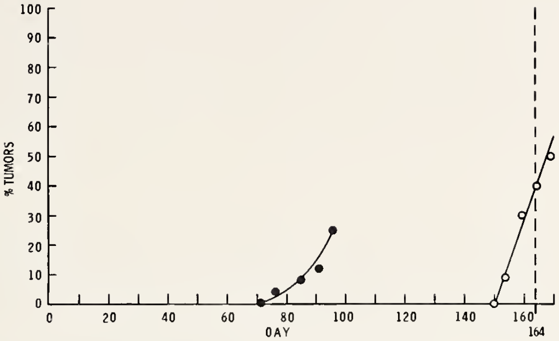
Our data showed good correlation between acute and chronic UVR exposure; the conditions that predisposed the animals to exaggerated acute injury also accelerated carcinogenesis.

How do increased temperature, increased humidity, and wind accelerate acute UV injury and promote carcinogenesis? We are not prepared to answer this question.

However, with regard to heat, some possibilities include a direct biochemical acceleration, a combination of thermal and UVR exposure, and a response to vasodilation.

Harber and Baer *(7)* showed a marked augmentation of the phototoxic reaction to topically applied 8-methoxypsoralen at high humidity. Although they attributed the effect to increased percutaneous absorption of the compound, our observations on the effect of humidity and UVR alone may partially explain their findings. Blum and Terus *(8)* stated that in 5 trials, application of water to the skin during sun exposure did not significantly affect the erythemal threshold, whereas Cattano*(9)* concluded that application of plastic occlusive dressings made the skin become more sensitive to sunlight. Kahn *(10)*, in discussing Cattano's article, reported a lower MED in 2 subjects whose arms had been immersed in water prior to irradiation. Perhaps hydration of the stratum corneum allows penetration of light that is normally scattered and reflected. Recently, Solan and Laden *(11)* have shown that light transmission values for isolated guinea pig stratum corneum was increased when it was exposed to water vapor, water, and certain organic liquids. This increased transmission increased with the refractive index of the liquid and reached a maximum at the refractive index of stratum corneum. To substantiate whether this was the mechanism involved, we conducted crossover studies in which animals were maintained and irradiated at low humidity and immediately placed in a high-humidity environment for observation as compared with animals maintained at high humidity, irradiated, and immediately placed in a low-humidity environment for observation. In these crossover studies, humidity was important before and during irradiation but had no effect in animals after irradiation. Therefore, it seems probable that increased transmission through a hydrated stratum corneum is responsible for exaggerated acute UV injury and promotion of carcinogenesis.

Recently, we performed an experiment in which hairless mice (10 in each group), maintained in the chamber at 90° F and 50% relative humidity, were given 20 MED of UVR from the Westinghouse FS-40 T12 sunlamp. At 72 hours of observation, damage was less in those exposed to wind while irradiated than in those irradiated without wind exposure. A possible explanation of this finding is that wind may offer a protective effect from a large single dose of UVR given over a short period, possibly by dehydrating the stratum corneum. After the 72-hour observation period, the same group of animals was given 3 MED daily for 1 week and at the end of this period, the animals exposed to wind and irradiation showed more damage than those receiving UVR alone. For longer periods, wind promotes UV damage and carcinogenesis, but we are not prepared to speculate on the pathophysiologic mechanisms involved.



TEXT-FIGURE 4.—Prevalence (mice with tumors/No. of surviving mice). *Solid circles* = UVR + 80% humidity; *open circles* = UVR + 20% humidity.

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Skin Cancer Epidemiology: Research Needs[1]

Joseph Scotto[2] *and* Thomas R. Fears[3]

ABSTRACT—The basic data currently being used to estimate and evaluate the dose–response relationship of UV-B and skin cancer are from a 6-month survey of four areas that participated in the TNCS, 1971–1972. Although most investigators from various fields of interest outside of cancer research, i.e. aviation, environmental ecology, physics, chemistry, and photobiology, etc., may admit an association between nonmelanoma skin cancer and UV-B exists, they point out that 1) the epidemiologic data currently available are too sparse and lack certain detail, such as exposure patterns and skin types, and 2) more data of this type are needed over a broad geographical range to allow for more precise measurements of the effects of stratospheric ozone depletion. They argue that the present relationships could change drastically with the addition even of a few more points (geographical locations) and that location-specific and demographic factors should be evaluated. Therefore, these data need to be updated and expanded to include more locations over a longer study period. The National Cancer Institute and the Environmental Protection Agency undertook a special skin cancer study from June 1, 1977 to May 31, 1978. The objectives of this study were: 1) to determine the incidence of nonmelanoma skin cancer (basal cell and squamous cell carcinomas) in various population groups within the United States, and 2) to ascertain and measure epidemiologic factors that may contribute toward the excess risk of nonmelanoma skin cancer in specific population groups.—Natl Cancer Inst Monogr 50: 169–177, 1978.

In light of the attention now given to skin cancer by the general public and by scientists, it is surprising to recall that in the past, skin cancer received little attention from most researchers. This situation appears to have come about because the usually fatal form of skin cancer, melanoma, is a rare cancer, whereas the more common nonmelanoma skin cancers are usually treated easily and successfully without hospitalization.

It was fortunate then that the NCI had completed in 1971–1972 its Special Skin Cancer Survey when concern was first expressed that certain human activities might result in increased UV radiation reaching the earth's surface. The data from the Special Survey are unique in that they are of sufficient detail to allow an appreciation of the size of the skin cancer problem and of sufficient scope to allow predictions of the increase in skin cancer incidence that would result from an increase in UV reaching the earth's surface.

The Environmental Protection Agency and the NCI have another epidemiologic study of skin cancer in progress currently (initiated June 1977). In the first part of this discussion, we will look at the considerations to be addressed by this study. In the second part, a detailed protocol of the study will be provided.

PROBLEMS WITH PREVIOUS DATA

The design of the new skin cancer survey was based on our experience and that of others in the analysis of existing skin cancer and UVR data. For illustration here, we will use the nonmelanoma study referred to earlier, which was conducted in Minneapolis-St. Paul, Iowa (entire state), San Francisco-Oakland, and Dallas-Ft. Worth, four locations which were part of the TNCS (*1*). We also refer to the UV measurements conducted for the Ultraviolet Radiation and Skin Cancer Working Group (*2*) at nine continental locations, including the four survey locations.

Several mathematical models have been proposed for the age distribution of cancer, but nonmelanoma data of consistent quality are available for only the four TNCS areas. Thus not enough data are available to allow a choice among the models. Cook, Doll, and Fellingham (*3*) examined data from cancer registries and showed that, for many cancers, a simple power function provided a good description of age-specific rates for ages 35–75 years. In work that appeared in the *American Journal of Epidemiology,* we use such a power function to describe the relation between age and age-specific skin cancer incidence rates but include the additional variables of UV insolation as measured in the Skin Cancer Working Group study:

$$P_{ij} = b\,(U_i)^c\,(A_j)^k$$

where P_{ij} = probability of developing skin cancer for the j^{th} age group at location i; U_i = annual total UV count at the i^{th} location; A_j is the midrange of the j^{th} age group; and b, c, and k are constants. If R_{ij} denotes the skin cancer incidence rate for the j^{th} age group at location i we have in logarithms:

$$ln\ R_{ij} = a + c\ ln\ U_i + k\ ln\ A_j + \epsilon_{ij}$$

where the ϵ_{ij}'s are normally and independently distributed with mean zero and variance equal to the inverse of the expected number of cases. All parameters are estimated by fitting regression lines with the method of least squares to the logarithm of the age-specific incidence rates associated with a weight equal to the observed number of cases.

Observe in table 1 that for both sexes the difference be-

Abbreviations: TNCS = Third National Cancer Survey; UV-B = carcinogenic UV rays; NCI = National Cancer Institute; UVR = UV radiation; BACER = Biological and Climatic Effects Research.

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[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

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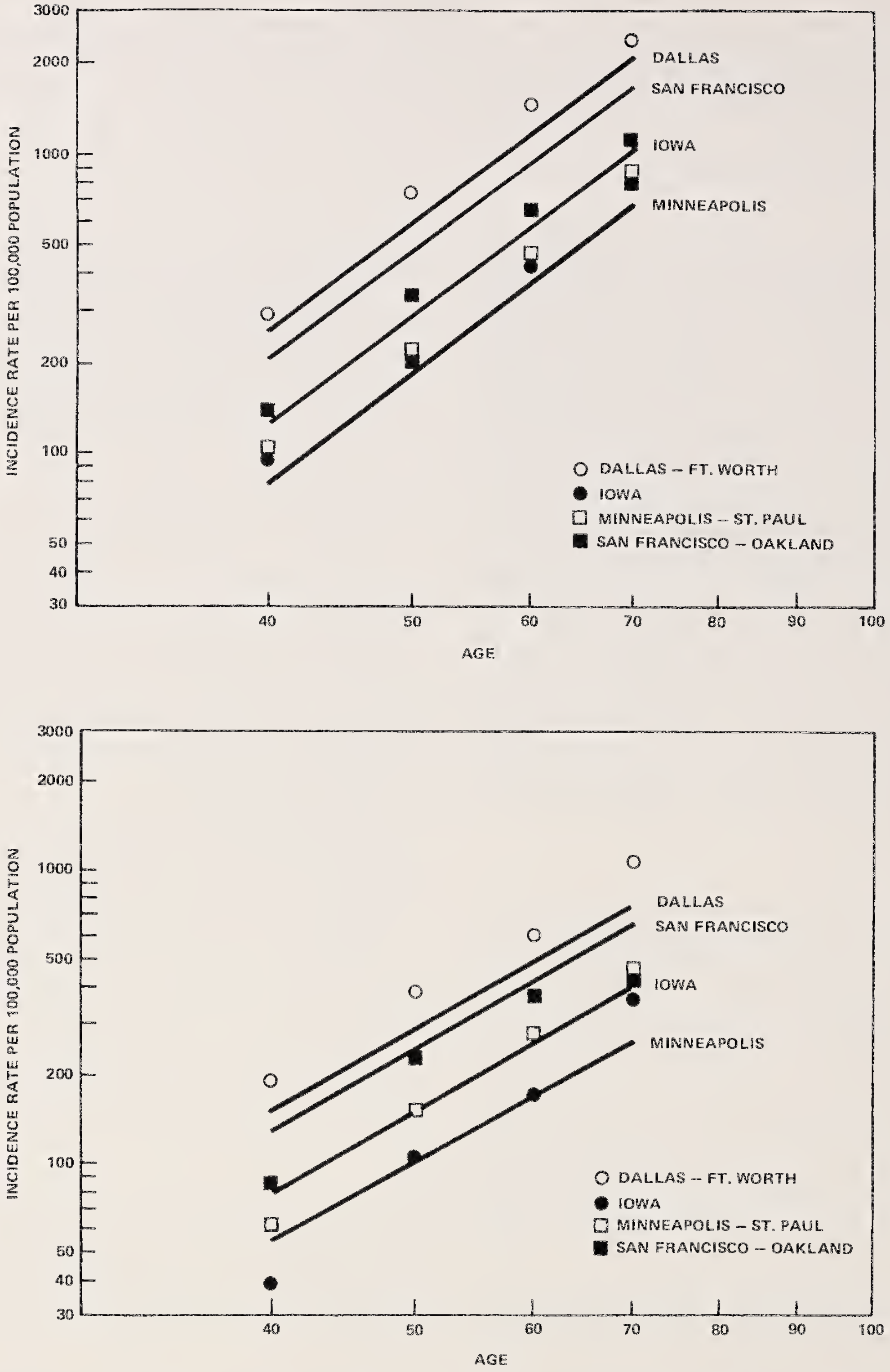
[3] Field Studies and Statistics Program, National Cancer Institute.



TEXT-FIGURE 1.—The ln incidence rate is compared with ln age for nonmelanoma skin cancers in males *(top)* and females *(bottom)*.

tween the regression coefficients (k vs. c) is small relative to the SD. These small differences suggest a common coefficient for ln age and ln UVR. If we regard the product of age and total annual exposure as a measure of lifetime exposure, then a common coefficient (k=c) suggests that skin cancer incidence is related to cumulative UV exposure.

The calculated ln rates are graphed against ln age in text-figure 1 for each of the four areas. The effect of different

TABLE 1.—*Summary of weighted least squares analysis*

Disease	Sex	a ± SD (constant)	k ± SD (age coefficient)	c ± SD (UV coefficient)
Nonmelanoma	Males	−50.6 ± 8.6	3.71 ± 0.45	2.96 ± 0.59
	Females	−41.3 ± 9.3	3.06 ± 0.49	2.45 ± 0.63

annual exposures at the locations is represented by the vertical distance between the parallel lines (i.e., the ln rate vs. ln age lines have different intercepts for different locations) depending on total annual UVR exposure at that location. The plot for females is similar but shows more scatter.

Of great importance to this discussion are the residuals, i.e., the differences between the observed and calculated values. The calculated values for Dallas-Ft. Worth and Minneapolis-St. Paul are generally lower than observed, whereas those for San Francisco-Oakland and Iowa are generally higher than observed. This indicates that location-specific variables other than UV insolation are important in the rates and possibly in our estimation.

Location variables that might affect skin cancer incidence rates are either intrinsic or extrinsic. Let us first consider extrinsic factors associated with skin cancer. We know some chemicals are capable of producing skin cancer, e.g., arsenic, shale oil, and other mineral oils (*4–8*). However, most of these compounds have only industrial applications, i.e., they are not in general use and therefore would not substantially alter skin cancer incidence rates. Further study is warranted.

The most important extrinsic factor is, of course, sunlight and, in particular, the erythema-producing sunlight (UV-B). Our behavior with respect to this factor, however, is important. Skin cancers usually appear on the head, neck, legs, and arms or those areas most exposed to sunlight (*9, 10*). The frequency of skin cancer is higher among individuals whose outdoor exposure is greatest (*11, 12*). Are the outdoor recreational exposure habits the same in Dallas and Minneapolis? Are more people employed outdoors in Iowa than in San Francisco? Just how dissimilar are the life-styles of the people living in the survey areas?

Of the intrinsic factors affecting skin cancer incidence, differences among races or populations of various ethnic backgrounds appear to be most important. Large differences in skin cancer incidence are observed between blacks and whites, with whites at a much higher risk than blacks.[4] Genetic effects have also been noted within the white populations (*14*), in which the fair-skinned Celtic people are at higher risk than darker skinned Caucasian populations. Just how strong is the impact of these intrinsic factors on our estimate(s) of the effect of UVR?

In summary, to improve our estimate of the relation between skin cancer rates and UV insolation, we need skin cancer survey data from as many different locations as possible, and to deal with location-specific factors, we need additional information on characteristics believed to influence the incidence of skin cancer. The new survey will attempt to get both sets of data.

PROPOSED SPECIAL SKIN CANCER PROGRAM

At the request of the BACER program, the NCI will conduct nonmelanoma skin cancer surveys in as many locations as possible within its ongoing Surveillance, Epidemiology and End Results program. This program, commonly referred to as the SEER program, follows in the footsteps of the TNCS, which ended in 1972. There are presently nine active locations within the contiguous United States (including a population base of more than 20 million, or roughly 10% of our total population) that provide incidence and follow-up data on all sites and types of cancers except nonmelanomas of the skin.

Plans are underway to include incidence reporting of nonmelanomas in seven of these locations. As with the earlier TNCS study, where Robertson-Berger UV meters were used at four locations, we have also asked that the meters be stationed in the new locations during the course of the epidemiologic survey.

Text-figure 2 is a map of the various locations providing NCI with skin cancer and UV data. The TNCS nonmelanoma locations are indicated by the finely dotted portions, except for San Francisco-Oakland and Atlanta (cross-hatched section), which is also included in the SEER program. The black dots identify the areas which provided UV data. The other six SEER locations where new nonmelanoma data will be included are indicated by slanted lines. They include: Seattle, Utah, New Mexico, New Orleans, Detroit, and Atlanta (cross-hatched). The locations that represent the other TNCS locations which have already reported skin melanoma data for the 3-year period 1969–1971 are designated by the vertical lines. Not shown on this map are several other areas presently providing melanoma data to the SEER program: Connecticut, Iowa, Hawaii, and Puerto Rico.

One may wonder why the SEER program did not include nonmelanoma in its basic protocol. Two basic reasons for this are: 1) Patients with nonmelanoma skin cancer are usually seen and treated in the doctor's office and are not hospitalized (overnight). Thus the prime source of data accession, the hospital chart, which provides complete and comprehensive information on virtually all other cancers, is not available. Therefore, a special effort must be made to collect these data from the physicians' office files (mostly dermatologists, pathologists, and radiotherapists). 2) The volume of data and the workload could be as much for skin cancer as for all other cancers combined. Thus a complete and comprehensive survey would require special personnel, operational facilities, and planning which increase costs considerably.

How much skin cancer do we expect in the new survey areas? Text-figure 3 depicts the relationship of skin cancers to all other cancer sites among whites in the four areas of the TNCS. In Minneapolis-St. Paul and Iowa, nonmelanoma rates were greater than 40% of the total for all other cancers; in San Francisco-Oakland, they amounted to about 50% of all other sites; and in the Dallas-Ft. Worth area, skin cancers were more numerous than all other cancers combined. Based on these data, we have estimated that the annual yield in all areas is as follows: northern SEER locations, Seattle and Detroit, 30–40% of all other cancer sites among whites (3,000 to 4,000 cases); San Francisco-Oakland and Utah

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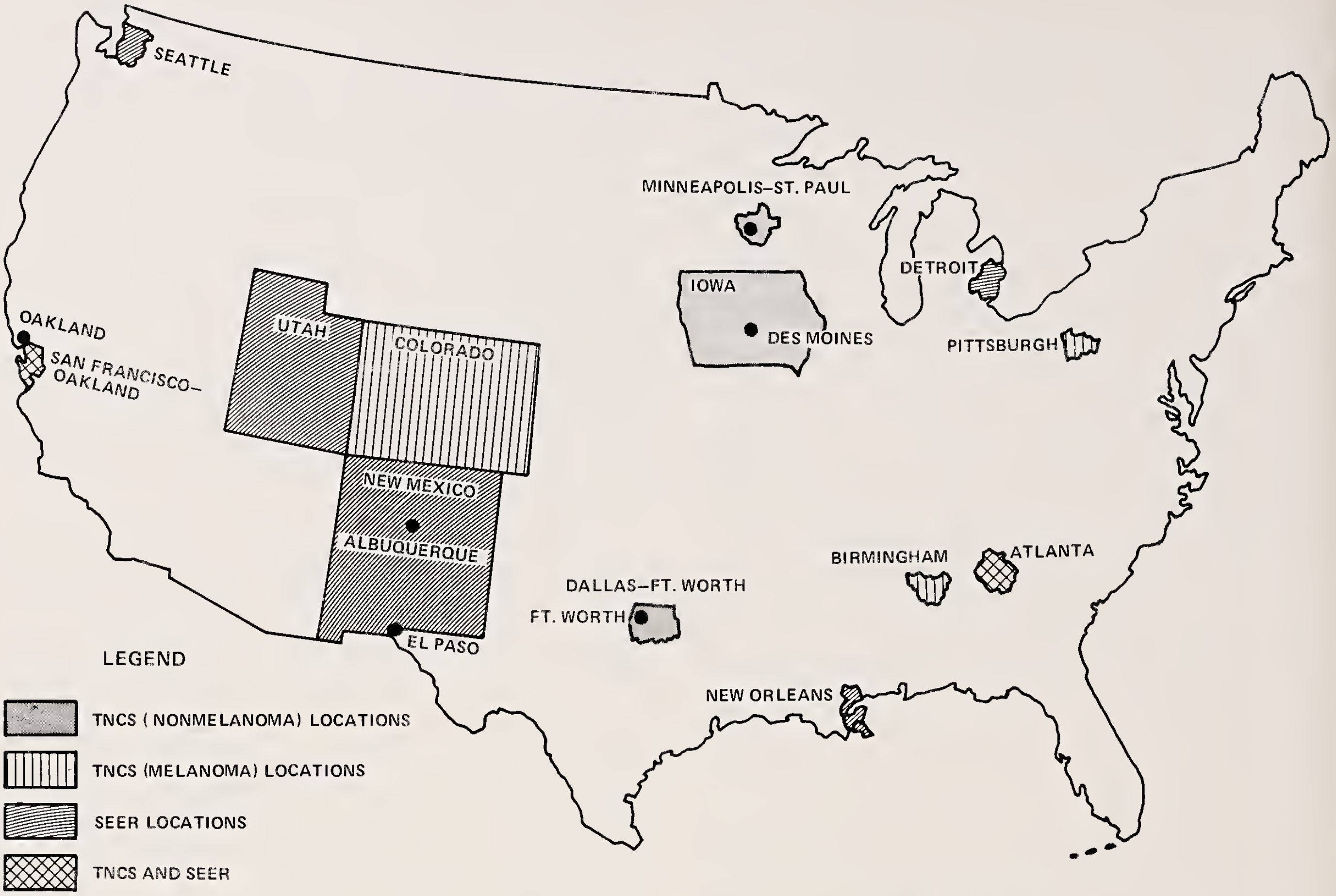
[4] Biometry Branch, NCI: Third National Cancer Survey: Advance Three-Year Report, 1969–71 (unpublished).



TEXT-FIGURE 2.—Skin cancer-UV measurement locations in the United States.

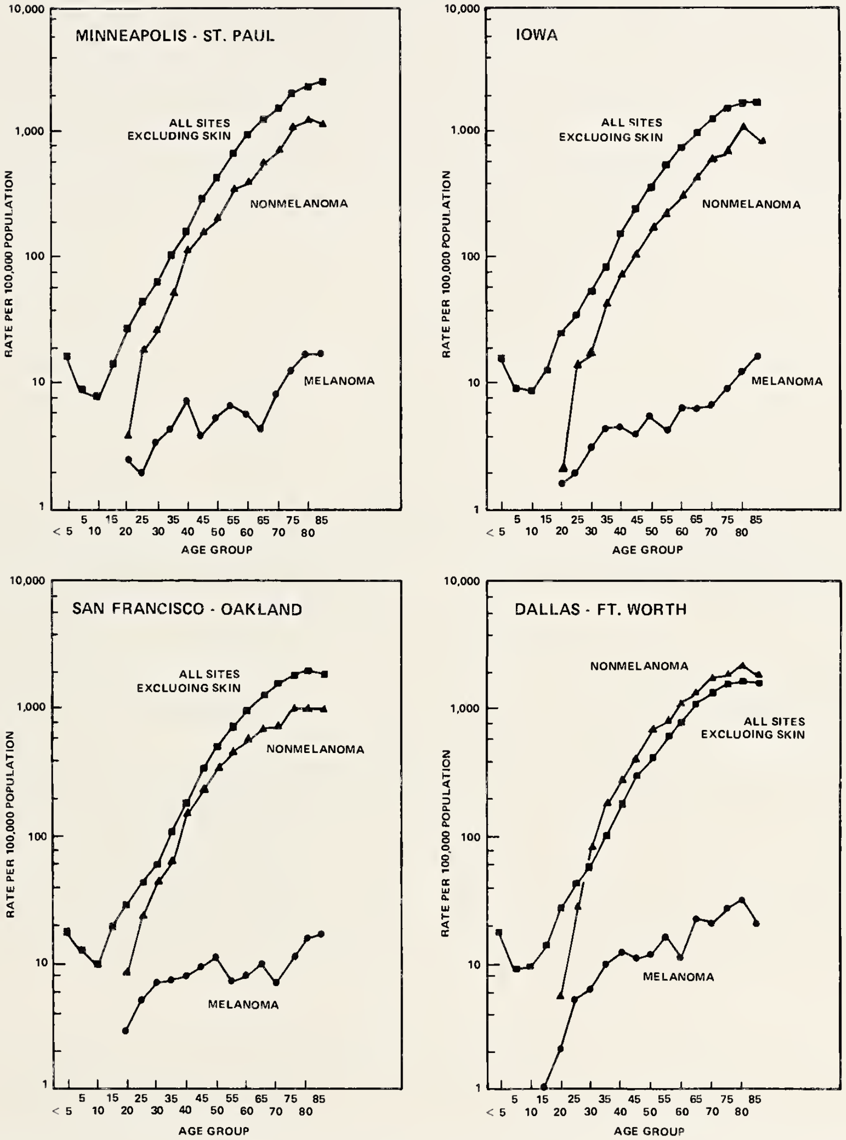
50% or more (6,000 to 9,000 in San Francisco and 3,000 to 4,000 in Utah); and Atlanta and New Orleans about 100% (5,000 in Atlanta and 2,000 in New Orleans). New Mexico may be hard to gauge because of the large numbers of Spanish-Americans (i.e., with darker skin pigmentation). We estimate that approximately 1,000 to 2,000 cases will be found annually in that location.

This new study plan has two phases: the incidence and the interview. The basic incidence or morbidity survey will be modeled after the earlier TNCS survey and will include a full 1-year study period. The overlapping area, San Francisco-Oakland, will provide trend estimates. Successful surveys in the other locations will provide supplementary points that can be used with the original four points in reducing some of the uncertainty in the present dose–response estimates.

Text figure 4 is a draft of the skin cancer abstract record that will be used by abstractors in the various field offices to submit reports of new cases of nonmelanoma skin cancer as of June 1, 1977. A minimum amount of information will be forwarded to the central office: case number, age at diagnosis, sex, race, date of diagnosis, anatomical site, cell type, method of diagnosis, and history of prior cancer. Names and addresses and other identifying information appearing on the top half of this form will remain in the field office. Only the portion below the dashed line will be forwarded for data processing.

Text-figure 5 shows the anatomical site chart detailing the specific sites. Physicians who are expected to see and treat skin cancer patients will be sent a supply of these charts, which they may keep, but data from them will not be processed. They will serve as a referral source for abstractors when a question arises about the exact anatomical site location.

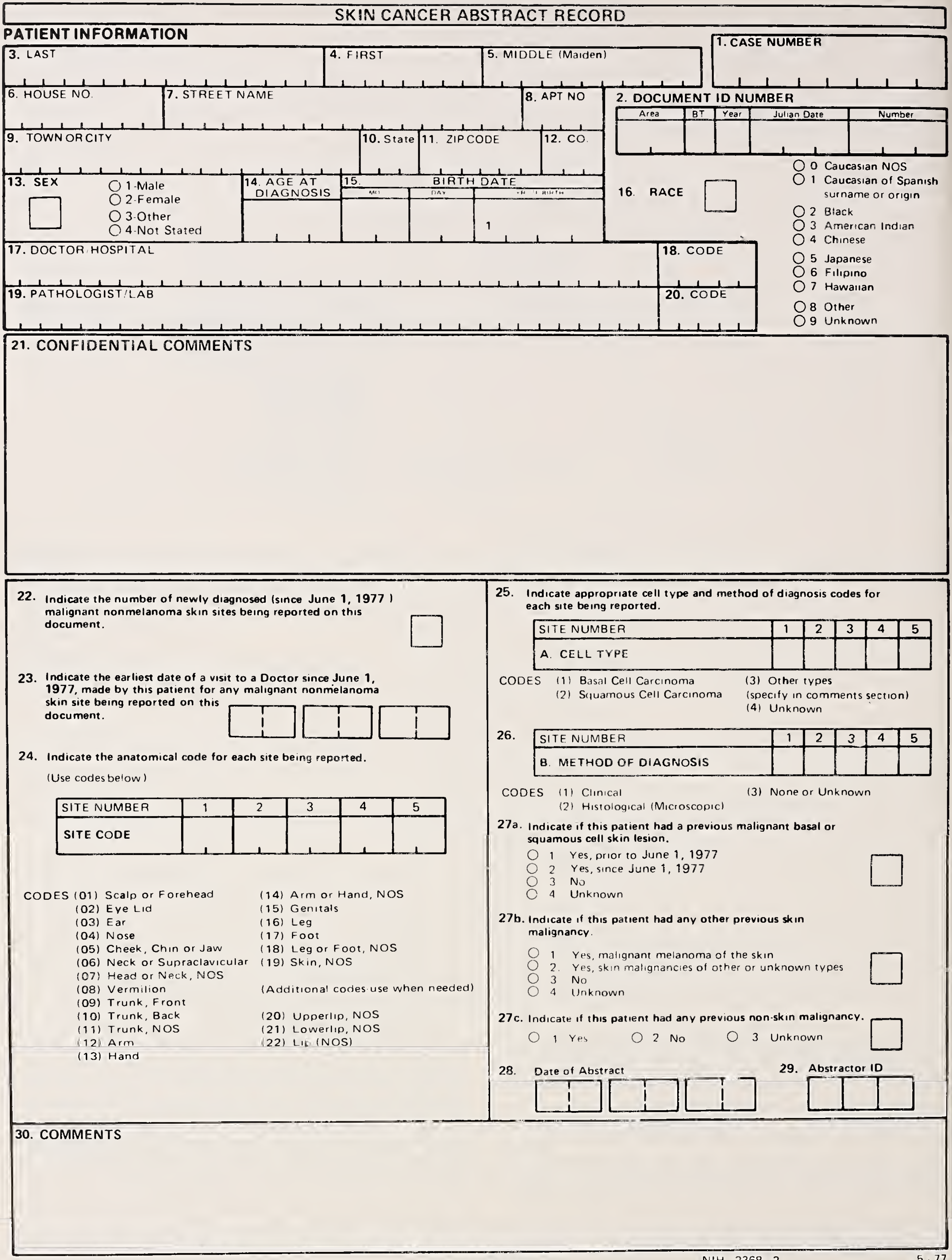
Figure 1 illustrates the telephone interview questionnaire. This entirely new phase of the study is intended to provide the epidemiologic data necessary to evaluate the degree of morbidity that may be related to high-risk factors. A short, one-page questionnaire requiring 10 minutes or less is required. After obtaining permission from the physician, a letter containing a copy of the questionnaire will be sent to a sample of patients and suitable controls. The letter will include a statement regarding the nature of the study and will advise the patient of his privacy rights. It also will mention that a member of the local field office will telephone at a reasonable time in the future to ask for an interview. The patients' free and informed consent will be obtained prior to any questions being asked. The epidemiologic items of interest included in the interview relate to two basic issues: 1) the individual's natural susceptibility, i.e., questions about eye color, hair color, ancestry or ethnic group, and skin complex-



TEXT-FIGURE 3.—Age-specific cancer incidence among whites in four areas of the United States.

ion; and 2) the individual's environmental susceptibility, i.e., questions about residence mobility, occupations, outdoor exposure and habits, exposure to materials which may harm or protect the skin, and other skin conditions.

To ensure that valid and reliable data will be obtained through the questionnaire, a pretest or pilot study is planned. The pilot test will determine whether specific questions are uniformly understood and whether the responses or results will be analytically useful in testing the hypotheses. The final format for the questionnaire, the sample size, and the methodology of selection of controls will be worked out with principal investigators at all participating SEER locations. Several BACER consultants have already made some useful suggestions that have been incorporated into the study, and we welcome additional comments and helpful hints from others.



SKIN CANCER ABSTRACT RECORD

PATIENT INFORMATION

1. CASE NUMBER

3. LAST
4. FIRST
5. MIDDLE (Maiden)
6. HOUSE NO.
7. STREET NAME
8. APT NO

2. DOCUMENT ID NUMBER

Area	BT	Year	Julian Date	Number

9. TOWN OR CITY
10. State
11. ZIP CODE
12. CO.

13. SEX ☐

○ 1-Male
○ 2-Female
○ 3-Other
○ 4-Not Stated

14. AGE AT DIAGNOSIS

15. BIRTH DATE

MO	DAY	YR OF BIRTH
		1

16. **RACE** ☐

○ 0 Caucasian NOS
○ 1 Caucasian of Spanish surname or origin
○ 2 Black
○ 3 American Indian
○ 4 Chinese
○ 5 Japanese
○ 6 Filipino
○ 7 Hawaiian
○ 8 Other
○ 9 Unknown

17. DOCTOR/HOSPITAL
18. CODE
19. PATHOLOGIST/LAB
20. CODE

21. CONFIDENTIAL COMMENTS

22. Indicate the number of newly diagnosed (since June 1, 1977) malignant nonmelanoma skin sites being reported on this document. ☐

23. Indicate the earliest date of a visit to a Doctor since June 1, 1977, made by this patient for any malignant nonmelanoma skin site being reported on this document. ☐☐ ☐☐ ☐☐

24. Indicate the anatomical code for each site being reported.

(Use codes below)

SITE NUMBER	1	2	3	4	5
SITE CODE					

CODES
(01) Scalp or Forehead
(02) Eye Lid
(03) Ear
(04) Nose
(05) Cheek, Chin or Jaw
(06) Neck or Supraclavicular
(07) Head or Neck, NOS
(08) Vermilion
(09) Trunk, Front
(10) Trunk, Back
(11) Trunk, NOS
(12) Arm
(13) Hand
(14) Arm or Hand, NOS
(15) Genitals
(16) Leg
(17) Foot
(18) Leg or Foot, NOS
(19) Skin, NOS

(Additional codes-use when needed)

(20) Upperlip, NOS
(21) Lowerlip, NOS
(22) Lip (NOS)

25. Indicate appropriate cell type and method of diagnosis codes for each site being reported.

SITE NUMBER	1	2	3	4	5
A. CELL TYPE					

CODES
(1) Basal Cell Carcinoma
(2) Squamous Cell Carcinoma
(3) Other types (specify in comments section)
(4) Unknown

26.

SITE NUMBER	1	2	3	4	5
B. METHOD OF DIAGNOSIS					

CODES
(1) Clinical
(2) Histological (Microscopic)
(3) None or Unknown

27a. Indicate if this patient had a previous malignant basal or squamous cell skin lesion. ☐

○ 1 Yes, prior to June 1, 1977
○ 2 Yes, since June 1, 1977
○ 3 No
○ 4 Unknown

27b. Indicate if this patient had any other previous skin malignancy. ☐

○ 1 Yes, malignant melanoma of the skin
○ 2. Yes, skin malignancies of other or unknown types
○ 3 No
○ 4 Unknown

27c. Indicate if this patient had any previous non-skin malignancy. ☐

○ 1 Yes ○ 2 No ○ 3 Unknown

28. Date of Abstract ☐☐ ☐☐ ☐☐

29. Abstractor ID ☐☐☐

30. COMMENTS

REGISTRY COPY 1
NIH - 2368 - 2
5 - 77

TEXT-FIGURE 4—Skin Cancer Abstract Record.

PHYSICIAN'S SKIN CANCER RECORD

REPORTING PHYSICIAN
PATIENT'S NAME
AGE
RACE
SEX

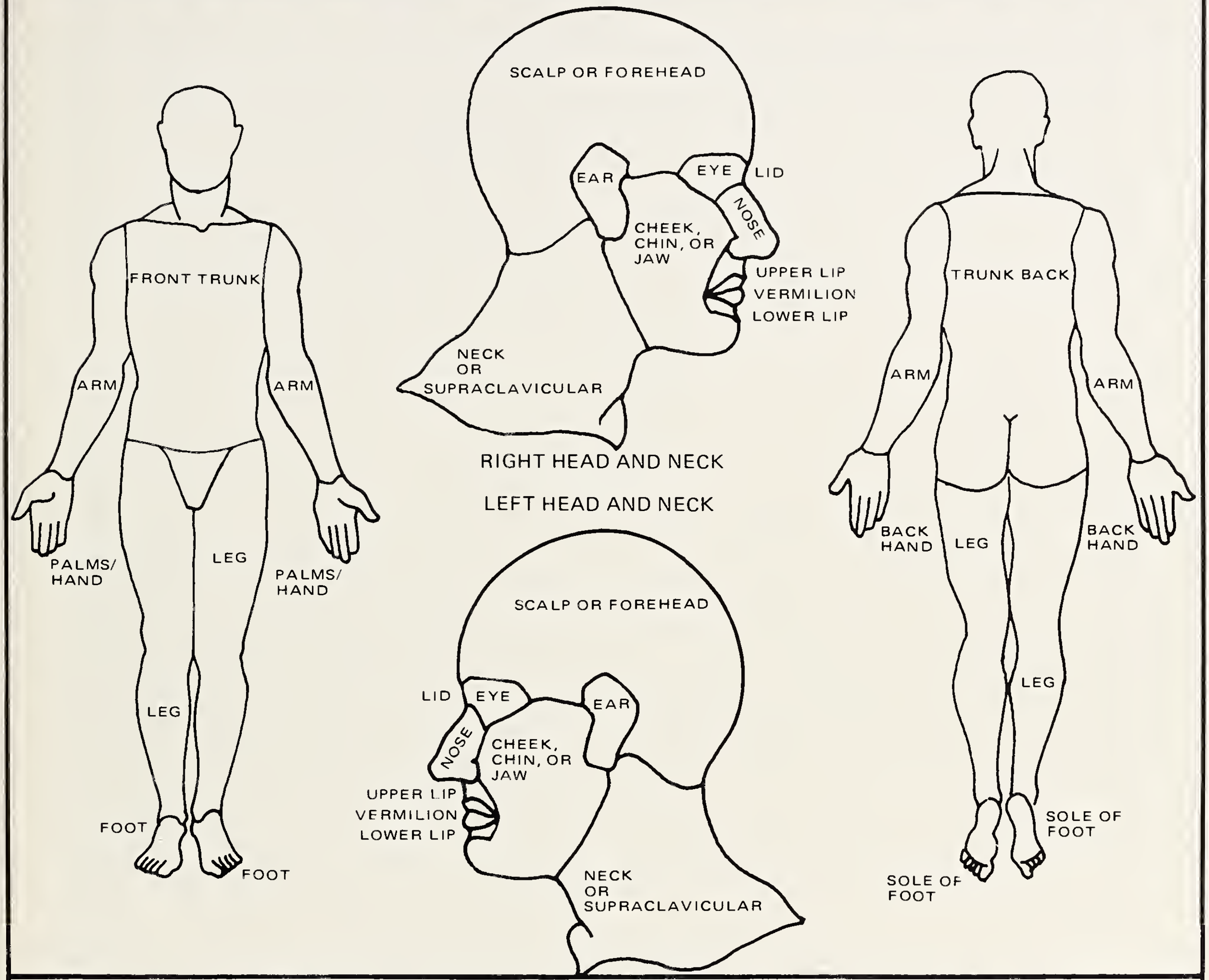
CELL TYPE
DATE OF DIAGNOSIS
COUNTY OF RESIDENCE

PLEASE OUTLINE THE AREA OF INVOLVEMENT FOR EACH PRIMARY, NEWLY DIAGNOSED SKIN CANCER SEEN JUNE 1, 1977.

FRONT TRUNK AND LIMBS

DO NOT CHART RECURRENCES.

BACK TRUNK AND LIMBS


SCALP OR FOREHEAD
EAR
EYE
LID
NOSE
CHEEK, CHIN, OR JAW
UPPER LIP
VERMILION
LOWER LIP
NECK OR SUPRACLAVICULAR
RIGHT HEAD AND NECK
LEFT HEAD AND NECK
SCALP OR FOREHEAD
LID
EYE
EAR
NOSE
CHEEK, CHIN, OR JAW
UPPER LIP
VERMILION
LOWER LIP
NECK OR SUPRACLAVICULAR
FRONT TRUNK
ARM
ARM
PALMS/ HAND
PALMS/ HAND
LEG
LEG
FOOT
FOOT
TRUNK BACK
ARM
ARM
BACK HAND
BACK HAND
LEG
LEG
SOLE OF FOOT
SOLE OF FOOT

PLEASE INDICATE IF THIS PATIENT HAD ANOTHER MALIGNANT NEOPLASM.

1. Indicate if this patient had a previous malignant basal or squamous cell skin lesion.
○ 1 Yes, prior to June 1, 1977
○ 2. Yes, since June 1, 1977
○ 3. No
○ 4. Unknown

2. Indicate if this patient had any other previous skin malignancy.
○ 1 Yes, malignant melanoma of the skin
○ 2 Yes, skin malignancies of other or unknown types
○ 3 No
○ 4 Unknown

3. Indicate if this patient had any previous non-skin malignancy.
○ 1 Yes ○ 2 No ○ 3 Unknown

NAME AND ADDRESS OF PATHOLOGIST OR LABORATORY WHERE MICROSCOPIC CONFIRMATION WAS MADE:

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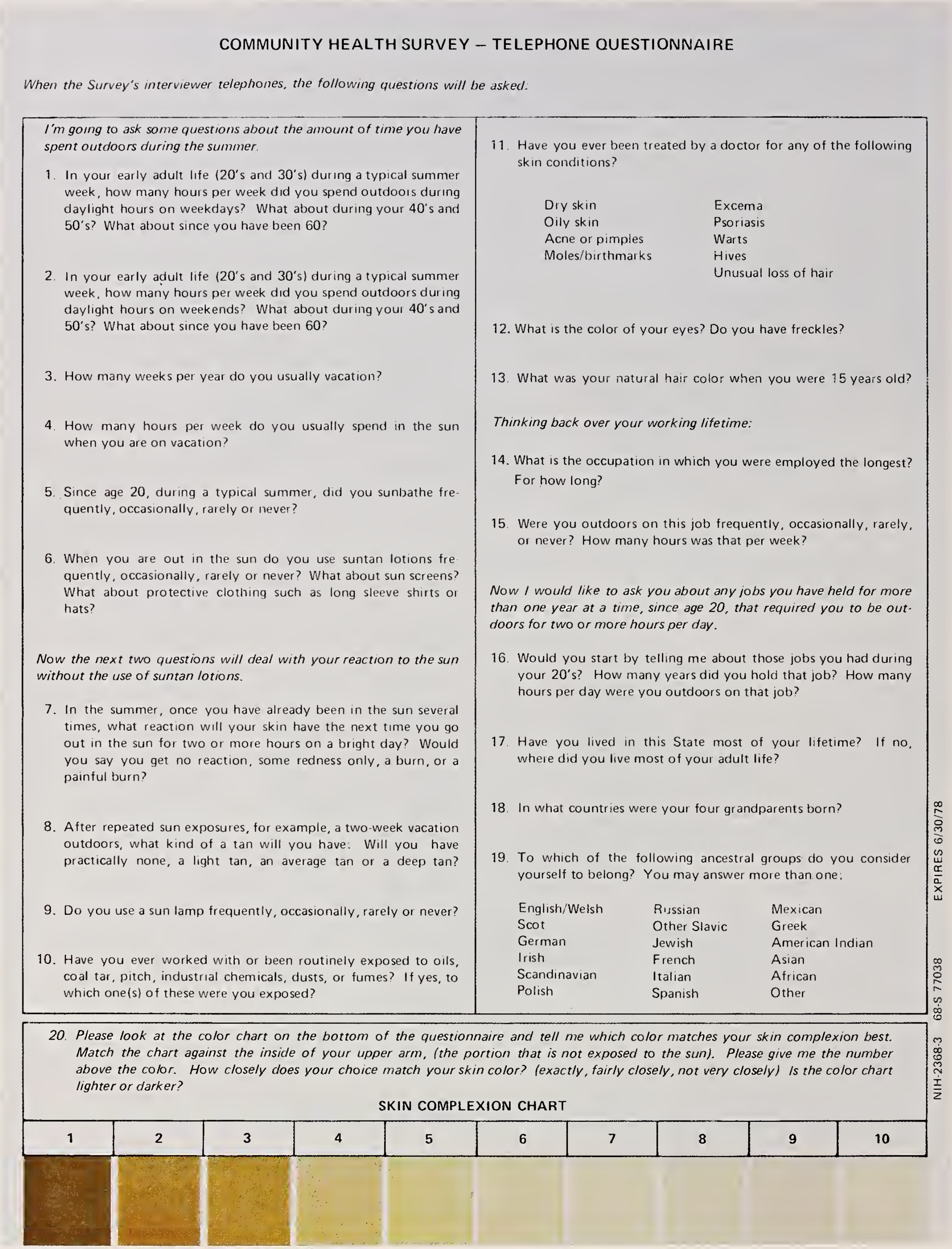
TEXT-FIGURE 5.—Physician's Skin Cancer Record.



COMMUNITY HEALTH SURVEY – TELEPHONE QUESTIONNAIRE

When the Survey's interviewer telephones, the following questions will be asked:

I'm going to ask some questions about the amount of time you have spent outdoors during the summer.

1. In your early adult life (20's and 30's) during a typical summer week, how many hours per week did you spend outdoors during daylight hours on weekdays? What about during your 40's and 50's? What about since you have been 60?
2. In your early adult life (20's and 30's) during a typical summer week, how many hours per week did you spend outdoors during daylight hours on weekends? What about during your 40's and 50's? What about since you have been 60?
3. How many weeks per year do you usually vacation?
4. How many hours per week do you usually spend in the sun when you are on vacation?
5. Since age 20, during a typical summer, did you sunbathe frequently, occasionally, rarely or never?
6. When you are out in the sun do you use suntan lotions frequently, occasionally, rarely or never? What about sun screens? What about protective clothing such as long sleeve shirts or hats?

Now the next two questions will deal with your reaction to the sun without the use of suntan lotions.

7. In the summer, once you have already been in the sun several times, what reaction will your skin have the next time you go out in the sun for two or more hours on a bright day? Would you say you get no reaction, some redness only, a burn, or a painful burn?
8. After repeated sun exposures, for example, a two-week vacation outdoors, what kind of a tan will you have: Will you have practically none, a light tan, an average tan or a deep tan?
9. Do you use a sun lamp frequently, occasionally, rarely or never?
10. Have you ever worked with or been routinely exposed to oils, coal tar, pitch, industrial chemicals, dusts, or fumes? If yes, to which one(s) of these were you exposed?
11. Have you ever been treated by a doctor for any of the following skin conditions?

Dry skin
Oily skin
Acne or pimples
Moles/birthmarks
Excema
Psoriasis
Warts
Hives
Unusual loss of hair

12. What is the color of your eyes? Do you have freckles?
13. What was your natural hair color when you were 15 years old?

Thinking back over your working lifetime:

14. What is the occupation in which you were employed the longest? For how long?
15. Were you outdoors on this job frequently, occasionally, rarely, or never? How many hours was that per week?

Now I would like to ask you about any jobs you have held for more than one year at a time, since age 20, that required you to be outdoors for two or more hours per day.

16. Would you start by telling me about those jobs you had during your 20's? How many years did you hold that job? How many hours per day were you outdoors on that job?
17. Have you lived in this State most of your lifetime? If no, where did you live most of your adult life?
18. In what countries were your four grandparents born?
19. To which of the following ancestral groups do you consider yourself to belong? You may answer more than one:

English/Welsh
Scot
German
Irish
Scandinavian
Polish
Russian
Other Slavic
Jewish
French
Italian
Spanish
Mexican
Greek
American Indian
Asian
African
Other

20. *Please look at the color chart on the bottom of the questionnaire and tell me which color matches your skin complexion best. Match the chart against the inside of your upper arm, (the portion that is not exposed to the sun). Please give me the number above the color. How closely does your choice match your skin color? (exactly, fairly closely, not very closely) Is the color chart lighter or darker?*

SKIN COMPLEXION CHART

1	2	3	4	5	6	7	8	9	10

NIH-2368-3 68-S 77038 EXPIRES 6/30/78

FIGURE 1.—Skin Cancer Epidemiology Telephone Questionnaire.

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Immunologic Aspects of Tumor Induction by Ultraviolet Radiation[1, 2]

Margaret L. Kripke *and* Michael S. Fisher[3]

ABSTRACT—Chronic treatment of mice with UVR not only induced skin cancer but also produced a systemic change that interfered with host resistance against these tumors. The studies leading to this conclusion were prompted by the discovery that most tumors induced in C3H mice by chronic UV irradiation were immunologically rejected following transplantation to normal syngeneic recipients. This raised the question of why these tumors were able to grow progressively in the autochthonous host without being destroyed immunologically. We found that after a short course of UV treatment, the UV-irradiated mice lost their ability to reject transplanted UV-induced tumors, even though such transplants were rejected by unirradiated animals. The growth of the transplanted tumors in UV-treated mice resulted from a systemic alteration in the animals that was induced by UV irradiation of the skin.—Natl Cancer Inst Monogr 50: 179–183, 1978.

Although carcinogenesis by UVR has been investigated extensively in mice (*1–3*), only recently has this tumor system come to the attention of immunologists. The first studies on the antigenicity of murine tumors induced by UV light were performed more than 10 years ago (*4, 5*) and provided a clue that this might be an immunologically interesting system. These skin tumors were shown to induce a vigorous immune response in syngeneic mice despite the fact that the primary tumors had an extremely long induction period. This finding was contrary to the popular generalization that tumors with long latent periods tend to have weak or negligible immunogenicity (*6*). Nonetheless, this apparent contradiction to current dogma remained unexplored until recently.

A second indication that this tumor system might have an immunologic association came from an unexpected and unfortunate source, that of renal transplant patients. Several investigators (*7–9*) reported an increased incidence and earlier age at onset of UV light-associated skin cancer in patients receiving chronic immunosuppressive therapy after a kidney transplant. It was postulated that immunosuppression might be responsible for, or at least contribute to, the accelerated appearance of skin tumors. Support for this hypothesis was recently provided by studies on hairless mice, which demonstrated that the induction of squamous cell carcinomas by UV light could be accelerated by treatment with some immunosuppressive agents (*9, 10*).

Because of this awakening interest in a possible relationship between the immune system and UV carcinogenesis, we had the opportunity to reinvestigate the immunogenicity of UV light-induced tumors. These experiments, which were never expected to produce anything more than confirmation of the original observations (*4, 5*), have led us through a series of unexpected results to the conclusion that the immune system does indeed participate in UV carcinogenesis.

IMMUNOLOGIC REJECTION OF UV-INDUCED TUMORS

Our first unexpected finding was that the transplantation of primary UV-induced tumors into syngeneic mice was usually unsuccessful. Actually, this phenomenon had been noted as early as 1939 (*2*), at which time, it was attributed to bacterial contamination. In our experience, however, this was such an unusual occurrence that we thought it worthy of further investigation. Therefore, we attempted to transplant these tumors in immunologically deficient hosts and discovered that they grew readily in immunosuppressed syngeneic mice. Furthermore, we demonstrated that the failure of tumors to grow in syngeneic recipients was due to immunologic rejection (*11*). This finding presented us with a paradoxical situation. If the UV-induced tumors were so antigenic that they were immunologically rejected by syngeneic mice, how could they survive and grow progressively in the primary host? This situation suggested that the developing tumors possessed some means of escaping detection or destruction in situ or that the primary host was altered in a way that favored tumor growth. Our subsequent studies have all centered around this question of why the primary host fails to reject its developing tumors.

SYSTEMIC EFFECT OF UVR

We first considered whether the inability of the host to reject its tumor was a local phenomenon, restricted to the site of the tumor, or whether it was expressed systemically. Primary fibrosarcomas induced by chronic UV treatment of C3Hf mice were surgically excised, cut into 1-mm^3 fragments, and retransplanted sc on the ventral (nonirradiated) side of the tumor donor. At the same time, tumor fragments were transplanted into groups of syngeneic age-matched normal animals and groups of recipients immunosuppressed by adult thymectomy and 450 R whole-body X-irradiation (*see* text-fig. 1). Although nearly all the tumor implants were rejected in normal recipients, most grew progressively in immunosuppressed mice, and all grew progressively in their primary host (*12*). This indicates that at the time when tu-

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Abbreviations: R = roentgens; UVR = ultraviolet radiation; DNCB = dinitrochlorobenzene; DMSO = dimethyl sulfoxide; LPS = lipopolysaccharide; MCA = 3-methylcholanthrene.

[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] Supported by Public Health Service contract N01-CO75380 with Litton Bionetics, Inc.

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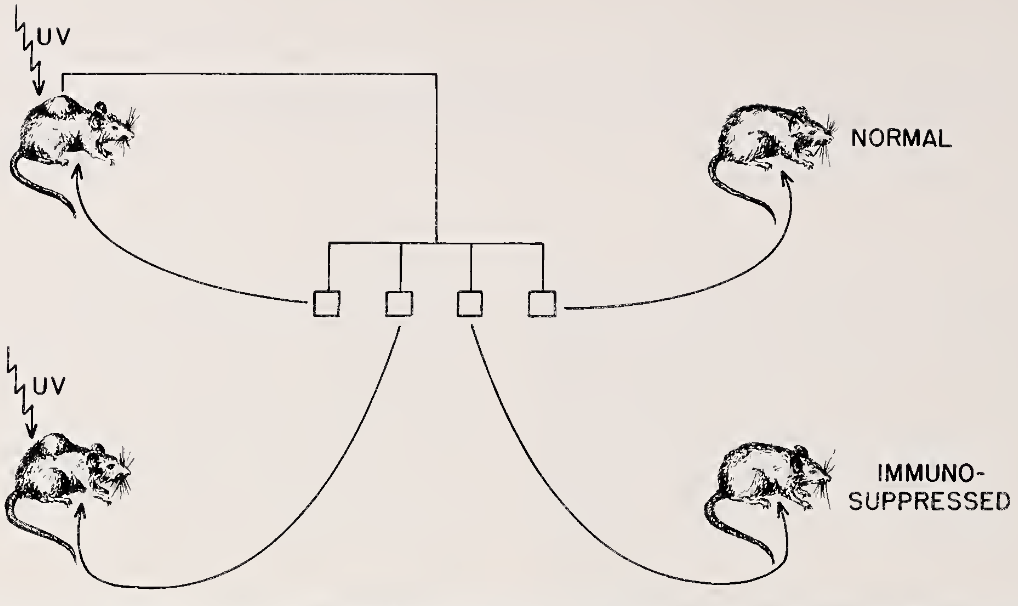

TEXT-FIGURE 1.—Scheme of tumor transplantation experiments. Primary tumors were removed, cut into 1-mm^3 fragments, and transplanted sc by trocar into various syngeneic recipients.

mors are detectable grossly, the primary host was systemically altered in a way that favors tumor growth.

We then tested the primary host for its ability to reject a UV-induced tumor taken from another animal. Since these tumors do not show antigenic cross-reactivity (*5, 11*), we wished to determine whether the lack of tumor rejection was restricted to the animal's tumor, or whether it extended to tumors induced by UVR in other syngeneic mice. Following transplantation, all the tumors grew progressively in a second primary host, which suggested that the failure of tumor rejection in the primary host is a systemic phenomenon, common to all UV-induced tumors (*12*).

This systemic alteration of the primary host could have been generated by either the presence of the tumor itself or by the chronic exposures to UVR. To distinguish between these possibilities, we transplanted fragments of UV-induced fibrosarcomas into groups of mice that had been UV irradiated for various lengths of time. We found that long before primary tumors appeared, the UV-treated mice were unable to resist the growth of UV-induced tumors implanted sc on the nonirradiated side of these animals, even though such transplants were rejected by unirradiated mice (*12*). The progressive growth of these tumors in UV-treated mice was due to a systemic alteration in the animals, induced by a short course of UV irradiation. Thus the UV irradiation somehow resulted in the lack of immunologic rejection of UV-induced tumors, and this absence of reactivity was expressed not only at the site of UV irradiation but systemically throughout the animal.

STUDIES ON THE MECHANISM OF THE SYSTEMIC ALTERATION

At this point, two questions of major importance arise for our studies of the immunologic aspects of UV carcinogenesis. The first is: How does UVR bring about the systemic effect we described? We have little information on this topic. At present, we know only that the target organ for UVR is the skin, not the eyes (*13*). Preliminary experiments suggest that the systemic alteration can be transferred to an unirradiated animal by a grafting of large areas of skin from UV-treated donors (Palaszynski E, Kripke M: Unpublished data). With studies of this kind, eventually we should be able to determine the location (e.g., epidermis, dermis, plasma) of photoproducts responsible for the lack of immunologic rejection.

The second question is: What is the mechanism behind the failure of tumor rejection? Several possibilities were considered. 1) The systemic alteration induced by UV irradiation might not be immunologic in nature. UVR could alter the host environment in a way that would permit the UV-induced tumors to outgrow or bypass the immune response. 2) Chronic irradiation might be generally immunosuppressive and thereby prevent tumor rejection. 3) UV irradiation may induce a specific unresponsiveness to the neoantigens of UV-transformed cells. Finally, UV treatment might selectively interfere with a subpopulation of cells that functions in host resistance against syngeneic and autochthonous tumors. In an effort to distinguish among these alternatives, we pursued three approaches. The first was to measure the general immunologic competence of mice during the course of UV irradiation, the second was to test lymphoid cells from UV-treated donors in vitro and in vivo in secondary hosts for reactivity against UV-induced tumors, and the third was to study host resistance to transplanted non-UV-induced syngeneic tumors.

Immune Responses of UV-Irradiated Mice

In these studies, we tested the general immune competence of UV-treated animals in various in vivo and in vitro assays (table 1). Inbred C3H(MTV$^-$) mice were irradiated with a bank of 6 Westinghouse FS40 sunlamps, which delivered an

TABLE 1.—*Immune responses tested*

- Tumor allograft rejection
  - UV-induced tumors
  - Spontaneous tumors
- Skin graft rejection
  - H-2 incompatible A → C3Hf
  - H-2 compatible $(AKR \times C3H^-)F_1 \rightarrow C3H^-$
  - Y-antigen C57BL♂ → C57BL♀
- Antibody production against sheep red blood cells
- Graft-versus-host reactivity
  - UV-treated donors
  - UV-treated recipients
- Delayed hypersensitivity to DNCB
- Inflammatory response
  - Footpad reaction to DMSO
  - Peritoneal exudate induction with thioglycollate
- Lymphocyte blastogenesis
  - Concanavalin A
  - LPS
- Macrophage functions
  - Phagocytosis
  - Tumoricidal activation with LPS or lymphokines

average dose rate of 2.8 joules/m^2/second over the wavelength range of 280–340 nm. The backs of the mice were shaved weekly, and they were exposed to the UV light for 1 hour, three times/week. The time required for tumor induction generally ranges from 22–42 weeks with this treatment schedule (*14*). Age-matched, unirradiated mice served as controls in these experiments.

Host Resistance

After 2 to 3 months of treatment, the UV-treated mice were unable to reject syngeneic UV-induced tumor transplants that were rejected by unirradiated controls. Nonetheless, they rejected both H-2 compatible and H-2 incompatible skin allografts in the same time span as the normal mice. The UV-irradiated mice also rejected spontaneous and UV-induced allogeneic tumors in a normal fashion. The primary hemagglutinin response to sheep erythrocytes was normal in these mice, as were the induction of a local graft-versus-host reaction with lymphoid cells from UV-irradiated donors and the induction of an inflammatory response to DMSO in the footpads of UV-treated mice. We observed an early transient depression of two reactions in the UV-treated hosts: delayed hypersensitivity to DNCB measured by footpad swelling, and the graft-versus-host reaction in UV-irradiated recipients measured by the popliteal lymph node weight gain assay. Both reactions returned to a normal level prior to the development of primary tumors (*15*).

Lymphocyte Reactions

We also investigated the ability of lymphocytes and macrophages from UV-irradiated mice to respond in vitro to a variety of nonspecific stimuli. In vitro blastogenesis induced by concanavalin A or LPS was not diminished in cultures of spleen or lymph node cells taken from donors treated with UVR for 1, 3, or 6 months. The cell numbers and proportion of cell types induced in a peritoneal exudate by ip injection of thioglycollate or pyran copolymer were also unaffected by chronic UV irradiation of the donor mice. Several macrophage functions were measured and also found to be normal. These were phagocytosis of carbon particles, phagocytosis of opsonized sheep erythrocytes, and the ability to kill tumor cells in culture following in vitro activation with lymphocyte mediators or LPS (*16*). Thus chronically irradiating the whole animal appears to affect only the response to syngeneic tumors, particularly those induced by UV light, and has no lasting systemic effect on the immune responses to common antigens.

IMMUNOLOGIC NATURE OF THE SYSTEMIC ALTERATION IN UV-IRRADIATED MICE

We then addressed the question of whether the systemic alteration in the UV-treated mice had an immunologic basis. First, UV-irradiated and unirradiated mice were challenged with a UV-induced tumor, and at various times thereafter, their spleen cells were tested in vitro for cytotoxic activity against the challenge tumor. Cytotoxic T-lymphocytes, which reacted specifically with the challenge tumor, were generated in the spleens of the unirradiated mice. In contrast, little or no cytotoxic activity could be detected in the spleens of UV-irradiated mice (*17*). This ruled out the possibility that the UV-irradiated mice were simply better hosts for the growth of the tumors and enabled the tumors to overcome an immune response. Rather, it suggested that no immune response was being made against these tumors in the UV-irradiated mice.

Another series of experiments tested the antitumor reactivity of lymphoid cells from UV-irradiated mice in various in vivo situations. These studies, described in detail in the succeeding paper (*18*), indicated that the failure of UV-irradiated mice to reject these tumors is due to an alteration in their lymphoid cell population (*19*).

GROWTH OF NON-UV-INDUCED SYNGENEIC TUMORS IN UV-IRRADIATED MICE

From the preceding studies, we could not determine whether the UV-induced alteration affected host reactivity against all syngeneic tumors or only those induced by UVR. We are attempting to distinguish between these possibilities by comparing the growth of non-UV-induced syngeneic tumors in UV-irradiated and age-matched control mice. Thus far, 4 tumors have been tested in C3H$^-$ mice and 3 in C57BL/6 mice. These are listed in table 2 (Kripke M, Lill P: Unpublished data). The mice had been UV-irradiated for 2–4 months prior to testing and were susceptible to challenge with UV-induced syngeneic tumors. Four of the 7 tumors tested grew equally well in UV-irradiated and normal mice. However, the growth of the B16 melanoma was dramatically accelerated in UV-treated C57BL mice (table 3), and 2 MCA-induced fibrosarcomas also exhibited more rapid growth in UV-treated animals but to a lesser extent than the B16 melanoma.

Thus UV irradiation does not appear to decrease host resistance against all syngeneic tumors per se or even against all syngeneic fibrosarcomas. Three tumors, all of dermal origin, did grow more rapidly in the UV-treated mice. However, we do not yet know whether this acceleration is immunologically mediated and stems from anti-

TABLE 2.—*Growth of syngeneic tumors in UV-irradiated mice*

Strain	Tumor	Increased growth rate or incidence in UV-treated mice
C57BL/6	Spontaneous melanoma (B16)	++
	Spontaneous fibrosarcoma	—
	MCA-induced fibrosarcoma (B6MCA)	+
C3H$^-$	Spontaneous mammary adenocarcinoma	—
	Spontaneous plasmacytoma (X5563)	—
	MCA-induced fibrosarcoma (113)	—
	MCA-induced fibrosarcoma (2231)	+

TABLE 3.—*Growth of B16 melanoma in UV-irradiated C57BL/6 mice*

Mice	Tumor incidence, wk after challenge[a]						
	2	3	4	5	6	8	10
UV-irradiated[b]	0/14[c]	6/14	10/14	11/13	12/13	12/13	12/13
Unirradiated	0/15	0/15	5/15	7/15	7/15	8/15	8/15

[a] Mice were given sc injections of 2.5×10^4 B16 cells.

[b] Mice were UV-irradiated for 1 hr three times/wk for 2 mo prior to tumor challenge.

[c] Values are cumulative No. of mice with palpable tumors/No. of mice challenged.

genic similarities among these and the UV-induced tumors, or whether this is due to physiologic effects of UV treatment on the host that produce a more favorable milieu for the growth of certain tumors.

SUMMARY AND CONCLUSIONS

Chronic UV irradiation has a profound effect on the immune response to skin tumors induced by UV light. Although many of these highly antigenic tumors are immunologically rejected by normal syngeneic mice, they grow progressively in UV-irradiated hosts. The mechanism by which UVR interferes with immunologic rejection is not completely understood, but several points have been elucidated. UVR impingement on the skin results in metabolic disturbances and cellular damage (*20*). Most likely, such altered cellular components serve as the intermediates between UV light and the immune system, but we have no evidence for this yet. Nonetheless, some type of intermediate alters the reactivity of those lymphoid cells with specificity for UV-induced tumor antigens that results in the failure of induction of an immune response against tumor antigens.

The finding that a selective immunologic defect precedes the appearance of UV-induced primary tumors suggests that an immune surveillance system may actually exist for cells transformed by UVR. If so, one would expect these transformed cells to develop into visible tumors only after the surveillance system was circumvented. This might explain the observation that regardless of the UV dose, there is a long latent period before primary skin tumors begin to appear (*3*). It is possible that this represents a period during which nascent tumor cells are immunologically eliminated. Thus tumors would begin to grow progressively only after this immunologic mechanism were depressed, either selectively by UV irradiation, or nonspecifically by aging or immunosuppressive therapy.

The finding that the growth of some non-UV-induced tumors is enhanced in UV-irradiated recipients raises an important issue. That is, UV light might act as a potentiating agent for the growth of certain tumors of diverse etiology. The enhanced development of the B16 melanoma is especially intriguing because of the suggestion that human melanoma is associated with UV exposure but indirectly (*21*). These studies also raise the possibility that UV irradiation could potentiate the growth of primary tumors induced by other carcinogenic agents.

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A Systemic Effect of Ultraviolet Irradiation and Its Relationship To Tumor Immunity[1, 2]

Michael S. Fisher[3]

ABSTRACT—Chronic irradiation of mice with UV light produces a systemic alteration that is immunologic in nature and may be due to the presence of specific suppressor lymphoid cells. The immunologic aspect of this systemic alteration was demonstrated by cell transfer experiments. Lymphoid cells from UV-treated donors were unable to confer tumor resistance to lethally X-irradiated and neonatal liver reconstituted recipients, whereas recipients given lymphoid cells from normal donors were resistant to a challenge with a syngeneic UV-induced tumor. Therefore, lymphoid cells from normal donors could mediate tumor rejection, but lymphoid cells from UV-irradiated donors could not. Furthermore, lymphoid cells from UV-treated donors suppressed the ability of lymphoid cells from normal donors to mediate syngeneic tumor rejection when mixed 1:1 before transfer into lethally X-irradiated recipients. This suppression was specific since all recipients resisted an allogeneic UV-induced tumor challenge. Serum transfer experiments failed to demonstrate any inactivating or suppressive substances in the serum of UV-treated animals. These findings suggested that UV-treated mice failed to reject UV-induced tumors because UV irradiation induced specific suppressor lymphoid cells that prevented the development of an immune response against these tumor antigens.—Natl Cancer Inst Monogr 50: 185–188, 1978.

Chronic irradiation of mice with UV light produces a systemic alteration that is detectable long before UV light-induced primary skin cancers begin to appear (*1*). This systemic alteration results in the failure of UV-irradiated mice to reject highly antigenic transplanted UV-induced tumors, which are immunologically rejected by nonirradiated syngeneic recipients. Although this systemic alteration prevents the rejection of syngeneic UV-induced tumors, it does not affect the ability of UV-irradiated mice to respond to H-2 incompatible skin and tumor allografts and other non-tumor-related antigens (*1, 2*).

These results suggested that UV treatment either altered the host environment in a way that permitted UV-induced tumors to outgrow an immune response or that it inactivated a subpopulation of lymphocytes, the specificity of which included the antigens on UV-induced tumors. To examine these possibilities, we performed parabiosis, cell transfer, and serum transfer experiments.

EXPERIMENTS

Growth of Transplanted Tumors in Parabiotic Mice

To establish a common circulation between mice, we joined the animals parabiotically according to the method of Eichwald et al. (*3*). Three weeks after surgery, each member of the pair was implanted with a syngeneic UV-induced tumor. At the conclusion of the experiment, each pair was tested for anastomosis by ip the injection of 5 mg trypan blue into 1 parabiont; the other member of the pair was checked 3 hours later for blue coloration. By this criterion, all pairs had a common circulation.

The purpose of this experiment was to determine whether the UV-induced systemic alteration was diminished by the nonirradiated member of the parabiotic pair or whether the UV-treated animal could transfer its susceptibility to the normal, nonirradiated partner. Data from table 1 show that when nonirradiated mice were joined in parabiosis, implants of a syngeneic UV-induced tumor grew in 6% of the partners (2/32 implants in 16 pairs). In parabiotic UV-irradiated mice, 93% of the challenge implants grew progressively (37/40 implants in 20 pairs). However, when nonirradiated mice were parabiotically joined to mice immunosuppressed by ATX followed by 450 R sublethal whole-body X-irradiation, only 7% (1/14) of the ATX mice developed progressively growing implants when challenged. However, when UV-treated mice were parabiotically joined to nonirradiated mice and then challenged, 74% (28/38) of the normal mice developed tumors and 79% (30/38) of the UV-treated partners developed progressively growing implants.

Thus establishing a common circulation between a regressor (nonirradiated) and a progressor (UV-treated) animal resulted in the conversion of the regressor partner to a progressor animal. These data also indicate that the systemic alteration in UV-treated animals is not equivalent to conventional immunosuppression, since the normal (regressor) mice converted the immunosuppressed (ATX) mice (progressors) to regressors. Furthermore, these data suggest that the conversion of the normal regressor to a progressor animal is not the result of a simple dilution of cells between each member of the pair, as seen by the ability of normal mice to mediate tumor rejection when joined parabiotically to ATX animals. Instead, the UV-treated mice have exerted some type of suppressive influence on their nonirradiated partners.

Cell Transfer Studies

These experiments were designed to answer the following questions: 1) Can lymphoid cells from UV-treated mice restore the ability of immunologically suppressed mice to reject

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Abbreviations: ATX = adult thymectomized; R = roentgens; FLATX = adult thymectomy plus restoration with syngeneic fetal liver cells.

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[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] Supported by Public Health Service contract N01-CO75380 with Litton Bionetics, Inc.

[3] Cancer Biology Program, Frederick Cancer Research Center, P.O. Box B, Frederick, Maryland 21701.

TABLE 1.—*Growth of transplanted tumors in parabiotic mice*[a]

Mice	Tumor incidence		
	Numerical[b]	Total[b]	Percent
Parabiotic partners			
Normal:normal	1/16:1/16	2/32	6
UV:UV	19/20:18/20	37/40	93
Normal:UV	28/38:30/38	58/76	76
Normal:ATX	0/14:1/14	1/28	4
ATX	8/20	8/20	40

[a] Animals were treated with 60 min of UV irradiation three times/wk for 3 mo prior to parabiosis.

[b] Values indicate No. of tumor-bearing mice/No. of mice challenged with tumor #1463.

tumor transplants? 2) Can lymphoid cells from nonirradiated mice or tumor-immune mice restore the ability of UV-irradiated mice to reject an implant of a UV-induced tumor?

UV-treated donors were irradiated for 3 months before their lymphoid cells were collected. Normal age-matched animals were also used as donors. Tumor-immune lymphoid cells were obtained from mice challenged with tumor #1591 sc and rechallenged 4 weeks later with the same tumor. Ten days after the second tumor challenge, the spleen and lymph node cells were used in the experiments. Some of the recipient mice were lethally X-irradiated (850 R) 24 hours before cell transfer. On the day following cell transfer, the mice were challenged with tumor #1591.

As shown in table 2, UV-treated mice given iv injections of 5×10^7 spleen and lymph node cells from normal animals were unaffected and remained susceptible to tumor challenge (10/10). Even when UV-treated mice were lethally X-irradiated before transfer of lymphoid cells from nonirradiated donors, they remained susceptible to tumor challenge (19/19). However, most animals receiving lethal X-irradiation and spleen and lymph node cells from normal donors were resistant to tumor challenge (5/18). When spleen and lymph node cells from UV-treated mice were transferred to lethally X-irradiated recipients, these animals could not mediate tumor rejection (17/17). Transfer of spleen and lymph node cells from tumor-immune donors to UV-treated mice resulted in partial restoration of tumor rejection (14/25), and injection of these cells into lethally X-irradiated, UV-treated mice produced even greater restoration (7/27).

TABLE 2.—*Growth of UV-induced tumors in mice given injections of lymphoid cells from either UV-treated, nonirradiated, or tumor-immune animals*[a]

Recipients	Spleen and lymph node cell donors			
	UV-treated	Normal	#1591 Tumor immune	Media control
Normal	0/10	—	—	1/25
850 R[b]	17/17	5/18	0/12	—
UV	—	10/10	14/25	26/26
UV + 850 R	16/17	19/19	7/27	—

[a] We gave 5×10^7 viable nucleated spleen and lymph node cells to recipients iv 24 hr before challenge. Both donors and recipients were treated with 60 min of UV irradiation three times/wk for 3 mo. Values indicate No. of mice with progressive tumor/No. challenged.

[b] 850 R whole-body X-irradiation was given 24 hr before lymphoid cell transfer.

These data show that at a dose of 5×10^7 spleen and lymph node cells, 1) lymphoid cells from UV-treated mice lack the capacity to mediate tumor rejection in vivo; 2) lymphoid cells from age-matched nonirradiated mice fail to mediate tumor rejection in UV-treated and UV-treated, lethally X-irradiated mice; and 3) tumor-immune lymphoid cells function to some extent in UV-treated mice and are highly effective when used to reconstitute UV-treated, lethally X-irradiated mice. These results suggest that chronic UV-irradiation creates a "suppressive environment," which is not altered by 850 R and which prevents the restoration of tumor rejection by transferred normal cells. Furthermore, cells from UV-irradiated mice are incapable of mediating tumor rejection, even when they are removed from the suppressive environment. The results also show that specifically immunized donors can transfer some degree of resistance to UV-treated recipients.

We performed similar experiments using recipients immunosuppressed by ATX, followed 4 weeks later by 850 R and restoration with syngeneic fetal liver cells. FLATX mice were used 1 week after reconstitution with fetal liver cells. As seen in table 3, lymphoid cells from normal animals restored the ability of FLATX recipients to reject a UV-induced tumor (4/10), compared with FLATX mice receiving media alone (18/19). Spleen and lymph node cells from tumor-immune animals also restored the ability of FLATX mice to reject a UV-induced tumor (2/20). However, spleen and lymph node cells from UV-irradiated animals failed to confer tumor resistance to FLATX recipients (20/20). These results parallel those obtained in mice immunosuppressed by lethal whole-body X-irradiation and again show that lymphoid cells from UV-treated mice are incapable of mediating tumor rejection in vivo even when removed from the UV-treated host.

In the previous experiments, the tumor challenges were given 24 hours after reconstitution of lethally X-irradiated recipients with spleen and lymph node cells. We then asked whether lymphoid cells given a recovery period in these recipients would now mediate tumor rejection. Recipients for cell transfer were first treated with 850 R, and 24 hours later they were reconstituted with spleen and lymph node cells. Animals failed to reject a syngeneic UV-induced tumor re-

TABLE 3.—*Growth of transplanted UV-induced tumors in immunosuppressed mice (FLATX)*[a]

Spleen and lymph node cell donors	Recipients	
	FLATX[b]	Normal
Media control	18/19 (95)	0/20
UV[c]	20/20 (100)	—
Normal	4/10 (40)	—
Tumor immune	2/20 (10)	—

[a] We gave 5×10^7 viable nucleated spleen and lymph node cells to recipients iv 24 hr before tumor challenge. Values indicate No. of mice with progressively growing implants/No. of mice challenged with tumor #1591.

[b] Mice were immunosuppressed by ATX and 850 R followed by restoration with approximately 2.5×10^7 syngeneic neonatal liver cells. *Numbers in parentheses* are percentages.

[c] Animals were treated with 60 min of UV irradiation three times/wk for 3 mo.

TABLE 4.—*Growth of UV-induced tumors in mice given injections of lymphoid cells*

Recipients	Interval between reconstitution and tumor challenge[a]: Day 1	Day 21
Normal	1/10	0/10
ATX	10/10	9/10
850 R + normal lymphoid cells[b]	2/8	2/9
850 R + UV lymphoid cells[c]	10/10	8/9
UV, 850 R + normal lymphoid cells	10/10	8/8
UV, 850 R + UV lymphoid cells	8/8	10/10

[a] Values indicate No. of mice with progressive tumor/No. challenged with tumor #1591.

[b] We gave 5×10^7 viable nucleated spleen and lymph node cells to recipients iv 24 hr after X-irradiation.

[c] Mice received 5×10^7 spleen and lymph node cells from donors irradiated with UV for 3 mo as above.

TABLE 5.—*Growth of UV-induced syngeneic or allogeneic tumors in mice following cell transfer*

Recipients: Strain	Treatment	UV-induced challenge tumor[a]: C3H$^-$ #1591	C57BL #112
C57BL	None	—	8/10
C3H$^-$	None	1/10	0/10
	850 R + normal lymphoid cells[b]	2/8	0/10
	850 R + UV lymphoid cells[c]	10/10	0/10
	UV, 850 R + normal lymphoid cells	10/10	0/9
	UV, 850 R + UV lymphoid cells	8/8	0/10
	850 R + normal and UV lymphoid cells[d]	10/14	1/14

[a] Values indicate no. of mice with progressive tumors/No. challenged.

[b] We gave 5×10^7 viable nucleated spleen and lymph node cells to recipients iv 24 hr after X-irradiation and 24 hr before challenge.

[c] Mice were given 5×10^7 spleen and lymph node cells from donors UV irradiated for 3 mo as above.

[d] As above, mice received iv 5×10^7 spleen and lymph node cells from nonirradiated and UV-irradiated donors.

gardless of whether they were challenged 1 day or 21 days after adoptive cell transfer (table 4). Once again, these results suggest that normal lymphoid cells are inactivated or blocked from mediating tumor rejection in UV-treated, lethally X-irradiated recipients and that lymphoid cells from UV-treated animals do not regain their ability to reject a syngeneic UV-induced tumor, even when removed from the UV-treated host and allowed a recovery period of 3 weeks in a normal environment.

SPECIFICITY AND MECHANISM OF IMMUNOSUPPRESSION SEEN IN CELL TRANSFER EXPERIMENTS

The failure of spleen and lymph node cells from UV-treated mice to confer tumor resistance to lethally X-irradiated and FLATX recipients suggests that the cells responsible for reactivity against UV-induced tumors were absent or inactivated, or a specific suppressor cell population was preventing the expression of reactivity. To test for the presence of suppressor cells and to test the specificity of the immunosuppression seen in the cell transfer experiment, we repeated this experiment. However, this time only half the mice were challenged with a syngeneic UV-induced tumor, whereas the other half were challenged with an allogeneic UV-induced tumor that grows progressively in its strain of origin. The data in table 5 show that although the mice given lymphoid cells from UV-irradiated donors were unable to reject the syngeneic tumor, they all rejected the allogeneic tumor transplants. In addition, when UV-irradiated mice were treated with 850 R and reconstituted with lymphoid cells from nonirradiated animals, they also failed to reject the syngeneic tumor, but they did reject the tumor allografts. Thus the immunosuppression was limited to UV-induced tumor antigens and did not include reactivity to major histocompatibility antigens.

A mixture of equal numbers of cells from UV-treated and nonirradiated donors mediated rejection of the allogeneic tumor (1/14) but failed to mediate rejection of the syngeneic tumor (10/14). These results suggest that specific suppressor cells are in the lymphoid population of UV-treated mice that prevent the development of a rejection response against UV-induced tumors. The suppressor cells seem to affect the induction rather than the expression of a tumor rejection response. If mice are immunized prior to UV treatment, they retain their ability to reject the immunizing tumor, even though they are susceptible to challenge with a second syngeneic UV-induced tumor (*1*). The specificity range of the suppressor cells includes the spectrum of neoantigens present on UV-transformed cells. This is an unusual situation because each UV-induced tumor has its tumor-specific transplantation antigen that does not cross-react in vivo with those of other UV-induced syngeneic tumors (*4, 5*). The specificity range does not include exogenous antigens such as histocompatibility antigens. This explanation is also consistent with the study in which normal animals became susceptible to tumor challenge after parabiosis with UV-treated partners.

The next question we asked was whether removal of an adherent cell population by glass wool filtration would restore the capacity for tumor rejection to lymphoid cells from UV-treated donors in a cell transfer experiment. Lymphoid cells from UV-treated animals were unable to confer resistance to a syngeneic UV-induced tumor (table 6) whether or not they were filtered through glass wool (10/10 vs. 10/10). However, glass wool-filtered cells from nonirradiated controls mediated tumor rejection in FLATX recipients (4/10). These data suggest that the effector cells involved in syngeneic, UV-induced tumor rejection are nonadherent lymphoid cells. Furthermore, these results show that suppressor cell activity cannot be removed by glass wool filtration.

EFFECT OF SERUM TRANSFER ON SYNGENEIC TUMOR GROWTH

To determine if a serum component might also play a role in the induction of unresponsiveness, we undertook

TABLE 6.—*Growth of UV-induced tumors in FLATX mice given injections of spleen and lymph node cells filtered through glass wool*

	FLATX recipients[a]	
Spleen and lymph node cell donors	Glass wool-filtered lymphoid cells	Lymphoid cells
Nonirradiated	4/10	—
UV[b]	10/10	10/10

[a] We gave 5×10^7 viable nucleated spleen and lymph node cells to recipients iv 24 hr before tumor challenge. Values indicate No. of mice with progressively growing implants/No. of mice challenged with tumor #1591.

[b] Animals were treated with 60 min of UV irradiation three times/wk for 3 mo.

TABLE 7.—*Growth of UV-induced tumors in nonirradiated mice treated with serum*

		Recipients[a]	
Experiment	Serum donor	Normal	ATX
I	UV 3 mo	2/10	—
	Nonirradiated	3/10	—
	None	0/10	9/10
II	UV 2 wk (5 treatments)	1/10	—
	Nonirradiated	3/10	—
	None	1/10	10/10

[a] Injection schedule was: 0.20 ml on days −3, −1, 0, 1, 3, 5, 7, 9, 11, and 13. Values indicate No. of mice with progressively growing implants/No. of mice challenged with tumor #1591.

serum transfer studies. Serum was collected from animals that had been UV-irradiated for 3 months and from nonirradiated age-matched mice. Animals were pretreated with serum for 3 days, implanted with a UV-induced tumor, and then given additional serum treatments. The data in table 7, experiment I, show that serum from mice treated with UV light for 3 months was unable to enhance the growth of a syngeneic UV-induced tumor. This suggests that the phenomenon observed in parabiosis was not due to the transfer of inactivating or suppressive substances in the serum of UV-treated animals to the nonirradiated member of the pair.

In a second experiment, serum for passive transfer was obtained from animals treated with UV for 10 days (5 treatments). This is the period in which acute skin damage occurs from UV-irradiation. However, as illustrated by the data in table 7, experiment II, this serum also failed to make nonirradiated animals susceptible to tumor challenge. These data also suggest that serum from the UV-treated parabiont did not alter the normal partner's ability to reject a UV-induced tumor implant. Thus it appears that the induction of the systemic alteration observed in parabiosis does not include a serum component. However, since the animals were challenged 3 weeks after parabiosis, it is possible that longer pretreatment periods with serum are necessary for manifestation of the systemic alteration. Nevertheless, when the same animals were challenged 3 months after initial serum treatment with a different, non-cross-reacting, UV-induced tumor, they retained their ability to mediate tumor rejection (Fisher MS: Unpublished data).

CONCLUSIONS

These results demonstrate that an immunologic defect in UV-irradiated mice precedes the development of primary skin cancers. Furthermore, this defect seems to be specific for the neoantigens on UV-transformed cells, since reactivity to allogeneic tumors is not altered. This defect was demonstrated in vivo by the transfer of lymphoid cells from UV-irradiated mice to lethally X-irradiated recipients. These recipients could not resist a later challenge with a syngeneic UV-induced tumor, whereas those mice given lymphoid cells from normal donors became resistant to tumor challenge.

The experiment in which lymphoid cells from nonirradiated and UV-treated mice were mixed before adoptive transfer suggests that specific suppressor cells are responsible for the lack of reactivity of UV-induced tumor antigens. The specificity of such suppression would presumably be restricted to antigens on UV-induced tumors, since UV-treated mice have normal immune responses to a number of exogenous antigens (*2*), and the transfer of susceptibility to challenge with syngeneic UV-induced tumors was accompanied by the transfer of resistance to an allogeneic UV-induced tumor.

How then do these highly antigenic UV-induced tumors become established and grow progressively in the primary host? These results suggest that UV irradiation induces unresponsiveness via the induction of suppressor cells that inhibit the development of a primary immune response against nascent skin tumors.

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Discussion: Possible Host Influences[1]

P. Forbes: The discussion will be opened by Dr. Gio Gori, followed by comments and questions concerning this set of papers.

G. Gori: The Co-chairman usually has the privilege of saying a few things out of context, and before we initiate the "Discussion," I would like to touch on some points that came to my mind during the presentations. Lest we forget, we should keep an eye out for man. After all, the human situation is the most important one, if the ultimate goal is to prevent cancer and UV-induced cancer in man. We should also keep in mind that prevention does not always require knowledge of the mechanism of action but can be satisfied with simple understanding of etiologic factors.

Dr. Fears and Mr. Scotto have told us about the need to explore the dose–response situation more closely. Again, the idea is to prevent exposure patterns that may increase the risk of cancer. We need to link cancer incidence with the solar irradiation at different geographical locations and to include a better understanding of exposure habits and conditions. Perhaps we could add a few more things to what Dr. Fears and Mr. Scotto have said. Genetics obviously plays an important role in UV carcinogenesis, and of course, their studies will take into account the genetics of pigmentation in particular, but I wonder whether there may be a need to develop simple field methods to test other manifestations of genetic variability, such as the capacity to repair or immune responsiveness.

Dr. Owens has alluded to other conditions that epidemiologists should look for: environmental conditions, heat, humidity, skin cover, and alterations of its index of refraction, etc. The clinical implications of what he was presenting and the epidemiologic implications are clear enough, in my opinion, even though it may be difficult to devise specific markers of easy field use for measuring these variables.

Dr. Kripke and Dr. Fisher suggested that we look at the immune competence in man. Should we develop a field test that could be used as a descriptor of susceptibility?

Clearly, other factors should also be considered, e.g., nutritional status. After all, we know that cellular differentiation of epithelial cells depends to a large extent on vitamin A or analog retinoids. Conditions of repair may be affected by nutrition. There is the question of antioxidants, natural or synthetic: selenium, vitamin E, perhaps vitamin C, although I think we are mostly speaking of hydrophobic compartments. Nutrition may also affect immune competence, cellular integrity, and skin features, such as the keratin layer and therefore permeability to radiation.

Stress may also be involved here. Mention of stress and the issue of corticosteroids and what that may do to the immune competence are beginning to be seen more frequently in the literature.

Concurrent infections, of course, are other conditions of susceptibility that we may have to study. Immunity may be modulated by concurrent infection: infections that depigment the skin, infections that may alter the integrity of the skin, the repair mechanism, etc. All these factors and others, together or alone, may affect the susceptibility.

I would like to add a few personal considerations. We need to look critically at any experiment and ask whether the results are of general validity and are likely to be of significance for man, or whether they describe simply a particular experimental circumstance. The experiments reported by Dr. Kripke and Dr. Fisher raise some questions from an evolutionary point of view. I believe it unlikely that the same set of circumstances observed in C3H mice may be true for man. Mice are nocturnal, burrowing animals that certainly have had different evolutionary precedents than man. Man, a diurnal animal, needs UVR if for nothing else than synthesis of vitamin D. In my opinion, the experimental situation observed in mice, if it occurs in man, will occur under exceptional circumstances, or perhaps at some distant end of the dose–response curve, perhaps in patients with XP or some similar disease.

What would happen if we had subjected these mice to a period of adaptation to UV light, rather than subjecting them to strong, periodic exposure to UVR, or to a different schedule of exposure? The issue of exposure schedule has troubled the epidemiologist and the etiologist in this field for a long time, and it will be taken into account in the epidemiologic studies that we heard are being set up currently.

Again, the ultimate goal is to control cancer in man and specifically cancer induced by UV radiation. This would be achieved by a more precise knowledge of the dose–response relationship and a more comprehensive knowledge of host predisposing factors.

I hope that we do not leave any stones unturned; that we do not put all our efforts in a few channels that may become fashionable rapidly; and that we dare to be imaginative and to extend our search to maximize our chances of finding the important elements in UV carcinogenesis, which as for other carcinogenic hazards, is likely to be a multifactorial event.

H. Blum: Dr. Fears, the effects of UVR are clearly cumulative, whether in mice or man, as my plotting shows. We have a theory, and it is substantiated by the data on man. It seems to me that unless we take this into account, we cannot properly apply statistics. If we treat these data linearly, as you have, then you must be in error, because the effect is cumulative: day after day, or year after year.

T. Fears: We did not treat the data as though they were linear. The estimate for c and the estimate for k were approximately the same. This suggests that the exponents c and k are the same. If we regard annual exposure times age as

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Abbreviation: UVR = ultraviolet radiation.

[1] Conducted at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

cumulative exposure, we are expressing the probability of a tumor as a function of cumulative exposure.

W. Clark: I have a question for Drs. Kripke and Fisher. I am so fascinated by this phenomenon that I do not care whether it occurs in man or not! I would like to know if this phenomenon is also produced if you induce the tumor through a port of UV.

If you irradiate a circle 2 cm in diameter on the back through a port, and you can induce exactly the same tumor, is this phenomenon the same?

M. Kripke: With this particular irradiation system, I do not know whether one can induce a tumor through such a port. The closest thing to this that we have done was to irradiate animals from which the hair was not shaved. This leaves the ears exposed, but the back receives only minimal exposure. Under those conditions, the systemic effect is still induced, but it takes much longer.

Clark: Also, is there a time prior to tumor development when this phenomenon appears? In other words, you waited until you got full-blown tumors that were large, ulcerative, spindle cell tumors. Prior to that, a lot of cutaneous change takes place. When in the course of tumor development does the systemic alteration occur?

Kripke: One of the slides that we showed illustrated the systemic phenomenon in animals exhibiting no gross or morphologic evidence of tumor formation. So before there was any evidence of visible tumors, the animals were systemically altered.

Now with short exposures, e.g., 1 week of treatment with UVR, the animals are resistant. Susceptibility to tumor challenge develops gradually during the course of irradiation, but nonetheless it occurs before primary tumors are visible.

J. Epstein: Did you biopsy the animal or do any histology at the stages when you were getting the effect, but no visible tumor was present?

Kripke: We have a box full of skin sections and you are welcome to them!

R. Latarjet: I would like to make a suggestion to Drs. Kripke and Fisher. When you speak of the suppressing environment, you suggest a diffuse action. I am a little skeptical, and I think you are also, about local action on circulating blood elements at the level of the superficial dermis. Then you suggest that some diffusible photoproduct could be responsible for the change in the environment. The most diffusible and perhaps abundant photoproduct is, I think, the superoxide ion, and since we now have purified superoxide dismutase available and we know that the mouse withstands treatment by that enzyme well, I would suggest that you prepare your UV-irradiated animals, not the tumor-bearing but those with the systemic change, in the presence of superoxide dismutase.

Kripke: Thank you. That sounds like a fascinating thing to try.

E. Scott: I would like to add some comments related to what Dr. Gori said and what Dr. Owens reported on the effects of temperature, humidity, and wind. We noticed earlier, when we were trying to analyze the data from the Third National Cancer Institute Survey and also the data on cancer mortality, that there were systemic deviations from the fitted curve. The observations from any one locality, e.g., San Francisco/Oakland, would be systematically too low for both sexes, each age group, both diseases (melanoma and nonmelanoma), incidence, and mortality. We tried to explain these systematic deviations by considering factors that would be special to that locality, such as life-style. Under life-style, we considered microclimate. We considered one index of microclimate as the number of hours in July in which the temperature was so extremely high that people would stay out of the sun, e.g., a climate such as in El Paso where people try to escape from the outdoors. Another index of microclimate was the number of hours when the temperature was high, but not extreme, and the humidity was high, when people tended to remove their clothing, go to the beach, etc.

Of course, a person's behavior may be affected by the temperature and humidity. However, we did find that both of our measures of microclimate had an effect on skin cancer. We could show this for our best data, in melanoma mortality, and this indicates that these interfering effects are present even there.

Secondly, I would like to ask Dr. Kripke a question. Would you regard the fact about melanoma and UV irradiation that you mentioned as an association between melanoma and UV?

Kripke: Of course I showed the slide deliberately, because I knew it would provoke a lot of discussion. It suggests the possibility that melanomas may "grow better" in UV-irradiated hosts, for some unknown reason, and that the association of UV light and melanoma may be indirect rather than causal. I have no way of knowing whether this transplantable mouse melanoma has any relationship to human melanoma and UV irradiation. It is not possible to generalize to that extent.

We tested only one mouse melanoma because these tumors are rare, and the ones that exist have been transplanted for many years. Thus it is not possible at present to perform a fair test of that association in animals.

Gori: Before we go on with the discussion, I want to say that I do not want to take the credit for having turned all of you from "mice–men" into "men–men!" My intention is to remind my colleagues of this particular need, and certainly I do not want to infer that studies such as Drs. Kripke and Fisher have reported should be avoided. I would hate to see 50 groups around the country jumping on this particular issue.

Clark: Fourteen!

Gori: I will go for 13.

T. Orme: I was convinced by Dr. Owen's talk that humidity and temperature are independent variables, but I have some questions about wind as an independent variable. Was hot moist air or cold dry air used in these wind experiments?

D. W. Owens: The wind experiments were repeated on several occasions; we irradiated Swiss albino mice for 4 weeks with one group exposed to wind and another protected from wind. The experiments were done at 80 and 20% humidity with the temperature at 32° C. We consistently reproduced the finding that wind promotes injury and delays recovery from UV injury. We did not see much difference between the group exposed to and the group protected from wind until we irradiated them for about 10 to 12 days; then the group exposed to UV alone began to recover much more quickly despite continued irradiation with the same dosage. This correlates grossly with thickening of the animal's skin. The animals exposed to wind and UV radiation and to high and low humidity, with the temperature in the range of 32° C, had delayed recovery.

I am unable to explain the mechanism involved with these effects from wind, although I think, with humidity, you could at least assume that it is probably due to increased transmission of UV through the hydrated stratum corneum. Although we do not know the mechanisms involved with wind, we repeated the study enough times to confirm our findings.

L. Thorington: My question is for Dr. Kripke and Dr. Fisher. In view of the work reported by Dr. Forbes, who found that adding long wavelength UV-A to the short UV-B had an apparent suppressing effect on the UV-B induction of cancer, do you feel that if you had used a light source simulating sunlight to deliver the UV-B dose, you would have different results?

Kripke: Not being a photobiologist and hardly knowing the difference between UV-A and UV-B, I would not know what to expect!

J. Robbins: One of the questions Dr. Clark asked you was not clear to me. Let me rephrase it, in the event it was not answered. Do you find at any time in your animals any increased resistance to transplantable UV-induced tumors before the suppressor effect occurs?

Kripke: If our hypothesis is correct, that we really have an immunologic problem and that it results from immunologic tolerance, there might be a period during this process when the animals are immunized, rather than tolerant. We are investigating this now by testing animals early in the course of irradiation for evidence of immunization. So far we have not found it, but our testing has not been extensive.

Robbins: Are you using the term "immunologic tolerance" in the strict sense of the word or are you referring to immunologic paralysis or to something entirely different from either of these phrases when you consider the suppressor function of a cell? Perhaps it would be best for you to define your definition of immunologic tolerance.

Kripke: Yes. Immunologic tolerance, by our current definition, does not imply mechanism. It means lack of immunologic reactivity. This may come about in several ways. One possibility is through clonal deletion of antigen-reactive cells. Another is the one that is supported by Dr. Fisher's experiment, i.e., the presence of an active suppressor mechanism. I think we need to review tests for suppressor cells other than that described by Dr. Fisher before we can conclude confidently that we are dealing with a suppressor mechanism. For the present, I prefer to use immunologic tolerance without implying mechanism.

Scott: I want to turn to another point related to the experiments reported by Dr. Forbes. I think it would be interesting to repeat those experiments with UV-B and UV-A with different dose rates and schedules.

Earlier experiments with other kinds of cancer, e.g., with urethan, have shown that carcinogenesis depends on the dose rate and schedule.

Forbes: Yes, a number of different schedules ought to be tested. The two experiments that I reported showed response rates that were different. Let me comment at the same time on the preamble to Dr. Thorington's question. This changes neither his question nor the answer that Dr. Kripke gave, but in his preamble, Dr. Thorington indicated that adding UV-A to the UV-B suppressed carcinogenesis; we did not show that. We showed no difference between UV-B alone and UV-B given simultaneously with UV-A; what was indicated in the experiment we did later was that when the time course was different (i.e., when the UV-B was given alone), giving the UV-A subsequently resulted in suppression. So in answer to your question, Dr. Scott, I think time course and dose ought to be studied, and they are going to be sizable experiments.

Gori: The implication is that different dynamics take place in the influence of UV on genetic or epigenetic targets, but I also believe that we should not lose sight of the different adaptation of different animals to tolerate or utilize UVR. What we are doing in one strain of mice may depend on their pigmentation, fur, or color of the fur. This seems to suggest again the need to be careful in transposing the results in these animals to what may happen in other animals when the adaptation may be totally different.

Scott: Oh, but that is just the point. The point is that we have a lot of evidence in man of a dose–rate effect, from what I have heard Dr. Fitzpatrick and many other persons say. Human cancer development depends strongly on whether the patient is getting his UV every day, or just on the weekends, or even once a year. We must try to get some understanding of these important factors. If we wait until a person comes into the doctor's office, it is too late.

R. Day: Dr. Kripke showed that the immune system seems to control the rate of growth of the tumor. What is the effect of the immune system on the induction of a tumor?

Kripke: The only way that this has been tested is by treatment of mice with immunosuppressive agents and determination of the effect on the rate of tumor induction. Such experiments have been reported by Nathanson and Forbes, by Koranda and Penn, and by Epstein. We have some evidence that if one gives an immunosuppressive agent to mice receiving UV irradiation, the tumors develop at a faster rate. All those experiments have been done on hairless mice. However, whether acceleration of carcinogenesis is due to the immunosuppressive effects of these agents, be they antilymphocyte serum, methotrexate, or azathioprine, is not known and cannot be assumed from these experiments. The animals are treated with agents that are immunosuppressive, but all these substances cause marked alterations of nonimmunologic processes as well. Therefore, the acceleration of carcinogenesis is not necessarily due to the immunosuppression: That is the limitation of these experiments.

N. Calvin: Dr. Kripke, can you transplant skin from a UV-irradiated mouse to a mouse genetically identical, which has not been irradiated, and will that mouse reject that skin?

Kripke: We have done a fairly extensive series of such experiments, i.e., transplanting UV-irradiated skin onto normal syngeneic recipients. Although some histologic evidence of rejection is noted, one does not see typical skin graft rejection. We have gone as far as performing first- and second-set grafts of pretumorous, UV-irradiated skin, and by this technique we cannot detect any consistent or convincing evidence of neoantigens in the skin before visible tumors appear.

Epstein: How long were the skin graft donors irradiated prior to graftings?

Kripke: These animals were irradiated up to 5 months, i.e., until they are just pretumorous.

Epstein: They should act antigenically, if any subclinical tumors were there, in that respect.

Kripke: Yes, although this may be a relatively insensitive means for detecting neoantigens.

Epstein: The immunosuppressive agents hydroxyurea and methotrexate given in low doses did not alter the onset or the growth of the tumors in our studies. Also, there is no definitive evidence that these agents affect cancer through immunosuppressive mechanisms when they are used in higher doses.

Robbins: The question I thought Dr. Day was asking, and if he did not ask it, I will, is: If you give a mouse the suppressor cells, is the induction time for recognizable clinical tumors reduced?

M. Fisher: We can alter the ability of mice to reject a transplanted UV-induced tumor by an iv injection of lymphoid cells from UV-treated donors, but we have not tested the effect of such a transfer on tumor induction.

M. Fry: On the one hand, we have the interesting results of Dr. Kripke, and on the other, the results are coming from experiments of skin cancer in nude mice; so now the picture for immunology and skin cancer has become more complicated. The results in the nude mice suggest that they are resistant to induction of skin tumors. I would like to hear Dr. Kripke's comments on that.

Do you intend to use hairless mice in some of these experiments because it would be easier to induce carcinoma in them?

If I understand the comments correctly, all these experiments were with sc transplantations of the tumors. Have you tried injecting cells iv and using a lung colony assay that would also help in the quantitation of your results?

Kripke: In your first question, you seem to be saying that nude mice are resistant to UV carcinogenesis. May I ask you with what that is compared?

Fry: I said that it appears difficult to assess, but as you know, the experiments have been done in two ways: comparing the effects in haired siblings and with comparison against hairless mice. Whichever way you look at it, the nude mouse appears to be more resistant to induction of skin tumors. Some of the data are still unpublished because of the problems of interpretation.

Kripke: In my opinion, the only appropriate control for this kind of an experiment is to compare UV carcinogenesis in the nude mouse that has been restored with a thymic graft. It is not appropriate to compare skin carcinogenesis in a nude mouse with a hairy heterozygous littermate, which is immunologically competent. No skin pathologist is going to let you get away with such a comparison!

To compare athymic mice with a different genetic background to other kinds of hairless mice is unfair. Until the appropriate comparison is made, one should reserve judgment.

Fry: Another point is that the experiments have not been done with UVR. At least I know of no experiments that have been done with UV induction of tumors in nude mice, which is, perhaps, what should be done. The experiments were all with chemical carcinogens so, of course, that is another factor. Reed[2] reported that the grafting of thymus gland or injection of thymus cells into nude mice made them susceptible to the induction of skin tumors by 7,12-dimethylbenz [*a*]-anthracene and Croton oil.

Kripke: Several experiments have been reported in which nude mice and heterozygous littermates have been injected with carcinogens. However, I think that it is likely that immunologic and nonimmunologic problems occur with nude mice. Although one can use these results to argue that immune surveillance does not influence chemical carcinogenesis, a number of other explanations are possible. Nude mice have ways of circumventing the immunologic defects; they are good at making other kinds of immune responses. For this reason, carcinogenesis experiments with nude mice must be interpretated with caution.

M. Hanna: I think another point about the nude mouse has to be kept in mind. The skin of the nude mouse is abnormal in many respects; aside from the immunologic aspects, I believe comparing UV carcinogenesis in nude mice to their normal counterparts would be like comparing apples and oranges.

I would like to comment on Dr. Gori's statement. It is always wise that these cautions be given to basic scientists. On the other hand, I think that it is unwise to assume that researchers do not look for analogies with man when they use these types of basic approaches in murine models.

It may be that the antigenic characteristics of the tumors are more important than the histologic types in a comparison of the murine system to man. That is one of the first things that immunologists look for in a tumor type when evaluating carcinogenesis and correlating it with immune response. These are, as Dr. Kripke pointed out, highly antigenic tumors, yet they do grow successfully. We have the same association in man. For example, the melanoma is the one human tumor from which several investigators have been able to isolate strong tumor-associated antigens. That is a most important point. The fact that the mouse burrows and has not adapted to UV light may simply be a factor that makes it a better model than man. The short-term induction of the tumors is probably a result of the fact that it has been protected from the light, but that does not necessarily mean that all its other metabolic and physiologic activities have been altered as a result of its burrowing.

Therefore, these are good cautions to make, but nevertheless, in the light of the fact that we cannot do many of these things in humans because of ethical reasons, we need to seek these types of experimental models. Also, I am really impressed by the data that were shown today by Dr. Fears and Mr. Scotto regarding the fact that 40% of all tumors in human populations were these highly antigenic skin tumors.

Gori: Thank you, Dr. Hanna. I was expecting somebody to come up and give me a lecture on this issue, because that usually happens. I make no apologies. I raise that point at every opportunity I find, but you do not have to overinterpret what I am saying.

Epstein: Dr. Fisher, since you developed sarcomas primarily, your schema presented your photoproduct in the epidermis where it really does not have to be. It could be a cellular alteration in the dermis that may not be easy to detect, even histologically.

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[2] Reed NB: Proceedings of the 1st International Workshop on Nude Mice (Rygaard J, Povlsen CO, eds).Stuttgart: Gustav Fischer Verlag, 1973, p 268

Fisher: That is entirely correct. When I spoke, I mentioned that the slide represented a much over-simplified version of the mechanisms responsible for unresponsiveness in the UV-treated animal. At this time, we do not have any idea about the nature of these photoproducts or their location, i.e., whether they are produced within the epidermis or the dermis.

Forbes: For the sake of those who might be interested, I am aware of one photocarcinogenesis study on nude mice involving thymus-reconstituted animals that is being done by G. J. Eaton at the Institute for Cancer Research in Philadelphia.[3]

Kripke: Dr. Fry, in answer to your other questions about our plans for repeating these experiments on hairless mice, which develop mainly squamous carcinomas, we have no plans for doing this. That is because most of the hairless mice in use now are on outbred backgrounds, and these animals are not useful to us for immunologic studies. We might reconsider if we could obtain a hairless mutant on an inbred background.

Your third question dealt with the transplantation of these tumors in sites other than the sc ones in UV-irradiated mice. We have not investigated this thoroughly, although this is one of the things we repeatedly said that we should study. I suspect, based on Dr. Fisher's experiments, that if this is truly immunologic tolerance, then it would make no difference where these tumors were inoculated. We should still see a differential growth in UV-irradiated and normal animals. We are in the process of investigating the growth of UV-induced tumors and also melanomas as artificial metastases following iv injection.

Orme: I would like to pose a question to anyone who is working with hairless *(hr/hr)* mice. Dr. Kripke indicated that she conducted many different tests to prove that the C3H mice she was using were immunologically competent and normal. Have the same types of experiments been done with the hairless mice? I saw an abstract by Schultz et al.[4] that indicated that hairless mice are deficient in some aspects of cellular immunity. Does anyone know anything about that?

Forbes: Most lines of hairless mice are not inbred and little is known about their immunologic parameters. I am aware of a few lines of inbred hairless animals, but until something can be done with those, we do not have much information.

Epstein: As Dr. Forbes said, we actually have many different types of hairless mice around. However we do not know which ones are alike and which are not. We know that the animals from The Jackson Laboratory are different from ours and from Dr. Forbes' mice. We did some transplantation studies several years ago but not enough of them to report, but our mice did, in fact, reject skin transplants. At least that part of their immune parameters was intact in our group of animals. I do not know whether it is true in other hairless mice. We are planning to do some skin-sensitizing and antibody-stimulation studies in the near future.

Gori: Well, we have had exciting demonstrations of science in the immunologic data presented. However, risking another admonition, I ask you to please consider that we may have lacked some perspective, because we have not begun to study all the possible host influences (the title of this session) nor, for that matter, environmental influences in general. I hope that we leave fortified by this intellectual stimulation to think actively of other opportunities besides immunology.

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[3] Eaton GJ, Custer RP, Crane AR: Effects of ultraviolet light on nude mice: Cutaneous carcinogenesis and possible leukemogenesis. Cancer 42:182–188, 1978

[4] Shultz LD, Heiniger J-J, Meier H, et al: Impaired cellular immunity and leukemogenesis. *In* 47th Annual Report, 1975–1976. Bar Harbor, Maine: The Jackson Laboratory, 1976, p 25

SESSION VI

Workshop and Summary

Workshop Chairman: Thomas B. Fitzpatrick

Panel: Wallace Clark
John H. Epstein
Thomas R. Fears
John A. H. Lee
Joseph Scotto
Arthur J. Sober
Frederick Urbach

Workshop: The Role of Ultraviolet Light in the Etiology of Human Melanoma[1, 2, 3]

The recent interest of biologists and physicians in skin cancer can be attributed in part to dramatic increases in incidence and death rates from melanoma. At the present time, the age-adjusted incidence rate of primary malignant melanoma of the skin in whites is 4.5/100,000. Melanoma is as common as Hodgkin's disease, and the increase in the incidence of melanoma, which some believe is related to increased sunlight exposure in susceptible Caucasoids, has been striking since World War II. Melanoma is a cancer that affects people in their productive span of 30 to 50 years, as Elwood and Lee have reported (*1*).

Mr. Joseph Scotto discussed the available information on melanoma incidence and mortality as a function of latitude, based on data gathered by the National Academy of Sciences, the Health and Nutrition Examination Survey, and the Third National Cancer Survey. Melanoma incidence and mortality increase with decreasing latitude; in contrast, no such latitude gradient exists for ocular melanomas (*2*).

At least three types of primary malignant melanoma of the skin in man are now known: lentigo maligna, superficial spreading, and nodular melanoma. Of these three types, lentigo maligna melanoma is clearly related to prolonged sun exposure, occurs in older people (around 70 years of age), and almost always appears on the head and neck. The development of the other two types is also believed related to sunlight exposure because they also occur on the head and neck (as lentigo maligna melanoma); in addition, they also develop on body sites (upper back, and on the legs in females) that are not completely covered but rarely occur in completely covered areas, e.g., bathing trunk area in males, bathing suit areas in females (*3*). Dr. Arthur J. Sober summarized the data from the Melanoma Clinical Cooperative Group of the United States, comprising 761 patients in which the exact anatomic location of each primary melanoma was depicted on human models. A nonuniform distribution is observed, with areas of highest density occurring on the head, neck, and back in males and females, and the lower leg in females. Spared areas are noted on the buttocks and thighs of males and females and on the breast and abdomen of females. This distribution of primary melanoma suggests that malignant melanomas infrequently develop on areas that are rarely, if ever, exposed to sunlight (*4*). The distribution of melanomas in the Melanoma Clinical Cooperative Group series is in agreement with the analysis of the Beardsmore study in Australia (*1*).

Clearly, the distribution of nonmelanoma skin cancer on the head and neck can be related to prolonged sunlight exposure. A higher incidence is noted in certain outdoor occupations (farmers, sailors, etc.), and evidence indicates that UV-B can induce nonmelanoma skin cancer in experimental albino animals. On the other hand, although melanoma has a high incidence on the head and neck, it does not occur more frequently in persons who have outdoor occupations; in fact, melanoma occurs more frequently in indoor professional and managerial persons who obtain intermittent exposures on weekends or vacations. Dr. Thomas Fears presented data that suggested that melanoma occurrence is related to intermittent solar exposure rather than total cumulative exposure. His data showed that melanoma does not have a strong relationship to age; the age coefficient with melanoma was small, whereas that with nonmelanoma skin cancer was larger. However, others believe that melanoma incidence does increase with age.

Dr. Wallace Clark suggested that the high incidence of melanoma on the backs of males and females and on the legs of females could be related to the special occurrence of targets of melanocytes, such as melanocytic nevi or special types of melanocytic foci, which are highly susceptible to sunlight exposure and develop into melanomas.

Unfortunately, malignant melanoma has not been induced by UV-B in any experimental animal models. Dr. John Epstein discussed a hairless mouse model in which it was possible to produce a benign blue nevus type of melanocytic neoplasm by a single application of DMBA. These tumors were then irradiated for several months, and some lesions developed locally invasive, malignant-appearing cells that spread to lymph nodes but did not cause death of the animals. Dr. Wallace Clark also discussed the induction in guinea pigs of a tumor similar to those human melanomas that start with a radial growth phase: superficial spreading melanoma and lentigo maligna melanoma. Clark used the method of prolonged topical application of DMBA to the Weiser-Maple guinea pig (*5*). The initial lesion is a pigmented macule that shows hyperplasia of epidermal melanocytes; subsequently, nodules develop within the macules, dermal invasion occurs, and metastases then develop in lymph nodes, lungs, spleen, and the gastrointestinal tract. Dr. Clark suggested that this induction in guinea pigs could possibly be accelerated with exposure to UV-B or that possibly this tumor could be induced with UV-B alone, if irradiation were given for a prolonged period.

Dr. Frederick Urbach agreed that the incidence of primary malignant melanoma is increasing in all parts of the world. He also agreed that lentigo maligna melanoma in man is probably induced by prolonged exposure to sunlight but that the inci-

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Abbreviations: UV-B = 280–320 nm of UV radiation; DMBA = 7,12-dimethylbenz[*a*]anthracene.

[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] The workshop was summarized by Drs. Thomas B. Fitzpatrick and Arthur J. Sober. Other panel members included Drs. Wallace H. Clark, John H. Epstein, Thomas Fears, John A. H. Lee, Mr. Joseph Scotto, and Frederick Urbach.

[3] *Address reprint requests to* Thomas B. Fitzpatrick, M.D. Department of Dermatology, Harvard University Medical School, Boston, Massachusetts 02114.

dence of this particular tumor is low (∼ 12%) and has not been increasing. He then commented on the evidence for the role of sunlight in the induction of the two more common types of melanomas: superficial spreading melanoma and nodular melanoma. He was not convinced of the latitude dependence of malignant melanoma and was also concerned that more melanomas occurred on the backs of males and females and the legs of females than on the head and neck, which is the most constantly exposed area of the body. Dr. Urbach postulated that if superficial spreading melanoma and nodular melanoma had any relation to UV exposure, sunlight plus some additional unknown factor must be involved. Dr. Epstein reminded the group that the development of melanoma is related to sunlight exposure in the human autosomal recessive disorder xeroderma pigmentosum.

Dr. Fitzpatrick, in defending a role for exposure to sunlight in the etiology of human melanomas, said:

1) Malignant melanomas are rare in the totally covered areas of the body, and the incidence is the same in these areas in white and black people.

2) Melanomas are rare in black people and when they occur, they do not arise on the backs of males and females or lower legs of females but on the soles of the feet or in the mucous membrane.

3) In agreement with Dr. Urbach and Dr. Clark, he concluded that the interaction of sunlight and some other factor must be implicated in the development of primary malignant melanoma in white persons and that this factor could be a special target lesion in these body sites (trunk and lower legs). He stated that his group at Harvard and that of Clark at Temple [now (1978) at the University of Pennsylvania] were now delineating a melanocytic lesion, which occurs on the back and does not appear to be a melanocytic nevus but a special type of pigmented lesion which could be a "target" lesion. These special types of pigmented lesions occur in certain families (*6*) or randomly in the general population as we determined at Harvard.

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Summary, Perspective, and Closing Remarks[1]

Frederick Urbach[2]

I have been asked to summarize and to place the exciting reports given at this conference in their proper perspective. It has always been my personal opinion that before trying to divine the future, it is useful to look back on what has gone before, so as to get the best perspective.

In any scientific field, indeed in all forms of endeavor, it is possible to identify milestones: Events that so dramatically changed our understanding of the subject or caused so major an alteration in the course of events may properly be called "quantum jumps." The discovery of the wheel in prehistoric times and, more recently, the unraveling of the helical structure of DNA were such milestones.

In the field of UVR-induced carcinogenesis, the first such milestones were the demonstrations by Findlay (artificial UVR sources) (*1*) and by Roffo (solar UVR) (*2*) that UVR of wavelengths shorter than 320 nm could cause skin cancer in experimental animals. These investigations provided experimental proof for the elegant (but circumstantial) hypotheses of Dubreuilh (*3*) and Unna (*4*) of the causal relationship of chronic sunlight exposure to the development of skin cancer in man. A decade and a half later, the classic studies of Blum and his colleagues (*5*) laid the foundation for quantitative photocarcinogenesis, and Latarjet and Lacassagne (personal communication) demonstrated the phenomenon of chemically enhanced photocarcinogenesis.

In the 1960's, Setlow and his collaborators discovered the "dark" repair mechanism of UVR-induced DNA damage (*6*), the existence of which Hollaender had suspected some time before (*7*). The importance of this (and a whole series of other DNA repair systems discovered in the ensuing years) for an understanding of the mechanism of both UVR and chemcial carcinogenesis was documented by, among others, Setlow, Latarjet, K. C. Smith, Regan, Radman, and many others (*8–11*).

This conference has been privileged to have had presented to it another "quantum jump"; i.e., the discovery by Margaret Kripke and Michael Fisher and their colleagues of the phenomenon that chronic irradiation with UVR produces a systemic change that interferes with host resistance against UVR-induced skin cancers, which are normally highly antigenic (*12*). Dr. Kripke's unique observation opens totally new avenues for the study of photocarcinogenesis and has most important implications for human skin carcinogenesis. It is also a classic example of serendipity.

This International Conference on Ultraviolet Carcinogenesis was called because of a demonstrated need to define the "state of the art" in a field that encompasses a broad spectrum of disciplines, ranging from molecular biology to human cancer epidemiology. The most immediate impetus has been the belated recognition that most human skin cancers are caused by environmental agents, and that, at least in white-skinned populations, solar UVR is one of the major environmental carcinogens (*13*). The probability that man-made contamination of the stratosphere may alter the protective ozone layer has raised the level of concern greatly in recent years (*14*).

The introductory papers of Urbach, Blum, Epstein, and Freeman describe the evidence for the assumption that human skin cancer is causally related to chronic UVR exposure and delineate the biologic events in experimental and human systems applicable to date.

Circumstantial evidence obtained from human epidemiologic studies strongly supports the role of sunlight and particularly the UV component in skin cancer formation. Experimental studies in animals indicate that UVR carcinogenesis begins with the initial exposure and that the effect of repeated doses accumulates (*5*). In addition, certain chemical agents (polycyclic hydrocarbons, psoralens, etc.) were found to have an additive effect on UVR carcinogenesis. Action spectra studies, albeit few, indicate that UVR of wavelengths less than 320 nm comprise the primary carcinogenic stimulus, although longer wavelengths (UV-A) may play an additive role. Detailed study of UV-irradiated mouse skin reveals alterations in epidermal cell morphology, DNA synthesis, cell transit time, and effects on the basement membrane and dermis.

In addition to the obvious importance of studies of UVR effects on tissues, cells, cell constituents, and molecules of biologic importance for a better understanding of human skin cancer, these investigations have served as models for all kinds of carcinogenesis and have pointed the way to the appreciation of DNA repair and mutagenesis to the general cancer problem.

The importance of the interactive effects of dose delivery (i.e., the need for a detailed description of the term "dose") was described by Forbes. Varying the dose rate (flux, fluence rate) while keeping the total amount of radiation given per increment (day) constant resulted in major differences in carcinogenic effectiveness, whereas fractionating the dose (keeping dose rate constant) had little effect.

Altering the spectrum slightly in a polychromatic UVR source also resulted in unpredicted effects. Thus the simplifying assumption of linear additivity of the carcinogenic effects of various UV wavelengths, which is implicit in most evaluations of the relationship of UV dose and carcinogenic re-

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Abbreviation: UVR = ultraviolet radiation.

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[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] Center for Photobiology, Skin and Cancer Hospital, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140.

sponse, is certainly not accurate and must be treated with great caution. Furthermore, the limits of reciprocity of photocarcinogenic effects must be studied much more extensively, since reciprocity in such experiments fails unpredictably and unexpectedly.

Fry et al. and Davies reported on the interaction of "light" and chemicals that can involve photophysical, photochemical, and photobiologic activity. Little information is available on the relationship between photosensitization of skin and cutaneous photocarcinogenesis, partly because there is so little understanding of the molecular lesions and subsequent cellular responses in common to phototoxicity and photocarcinogenesis. Fry et al. found that there was *no* quantitative correlation between psoralen–DNA cross-link production and tumorigenesis, thus negating a recently proposed explanation suggested for the psoralen-enhanced photocarcinogenesis phenomenon. They also showed that two hairless mouse strains had markedly different susceptibility to psoralen–UV-A-induced skin cancer development, a reminder that the genetic makeup of the test animal often seriously influences the outcome of experiments.

Davies reviewed the types of interactions between light and chemicals, their biologic effects, and the possible relevance to practical control measures.

Stenbäck reported extensively on the microscopic and ultrastructural features of the various types of tumors produced in human and animal skin, including the changes that take place during progression from early to fully developed tumors. Mondal and Heidelberger then presented their findings on in vitro oncogenic transformation. Their data showed that UVR acted as a pure initiator in the process of in vitro oncogenic transformation and required a promoter to complete the process. DiPaolo presented the results of his extensive studies, with different results.

Various aspects of the most recent studies on pathways of DNA repair, their role in mutagenesis and cell transformation, and the importance of these DNA repair systems for the understanding of carcinogenesis were discussed by Smith, Miller, Radman, Sutherland, Latarjet, Regan, and Lytle. The great variety of repair mechanisms discovered thus far is impressive: There clearly are multiple pathways of repair at the command of the cell. It appears likely that mutagenesis also results from error-prone DNA repair. This suggestion is strongly supported by the studies of Zajdela and Latarjet, in which caffeine and theophylline (inhibitors of error-prone repair) strikingly reduced the incidence of UVR-induced skin cancers in intact, irradiated mouse skin. The extensive work on DNA repair mechanisms in bacteria, including detailed chemical mapping of DNA base lesions (Miller) and the elucidation of molecular mechanisms of UV-induced mutagenesis, is applicable to events occurring in mammalian cells (Radman).

Sutherland's discovery of photoreactivating enzyme in mammalian cells may help to explain some of the peculiar photocarcinogenic responses to polychromatic light sources and some of the failures of reciprocity.

One of the keystones on which the assumption that misrepair of DNA is related to skin carcinogenesis in man is based on the observations that cells of patients with xeroderma pigmentosum, who develop skin cancer in light-exposed areas in childhood, often are deficient in DNA repair (*15*). Regan and Carrier, who, with Setlow, pioneered studies in this field, reviewed the evidence for repair deficiencies in xeroderma pigmentosum cells and reported on newer findings.

Using UV-irradiated viruses, particularly herpes virus, Lytle showed that radiation-enhanced reactivation in mammalian cells is inducible, an observation that may be important to the basic mechanism of carcinogenesis.

One of the major contributions to this conference was a workshop entitled "Production and Measurement of Ultraviolet Light." The introductory remarks on UV sources, measuring devices, and particularly standardization of dosimetric quantities sparked a lively discussion. Clearly, the subject is a difficult one, and as on other occasions, no complete agreement was reached on terminology. Of particular interest was the presentation of a photochemical personal UV-B dosimeter by Magnus and a description of electronic integrating dosimeters by Berger and Latarjet. The problem of detailed practical calibration of UV light sources was also discussed, and as usual, it was found difficult.

That environmental factors in addition to UVR affect skin carcinogenesis was described by Owens, who studied the effect of concomitant variation in heat, humidity, and wind. Not unexpectedly, increased temperature and humidity significantly facilitated UV skin carcinogenesis. The augmenting effect of wind, however, is as yet unexplained.

More detailed epidemiologic and statistical data on the incidence of and distribution of human nonmelanoma and melanoma skin cancer were discussed by Fears and Scotto and in a workshop chaired by Fitzpatrick.

Further evidence for a strong relationship of nonmelanoma skin cancer in white-skinned people in the United States is accumulating. Fears and Scotto showed that with existing data, a simple power relationship can be used to provide a good description of the UV–age–nonmelanoma skin cancer incidence relationships. These cancers are strongly related to cumulative UV exposure, a finding that corresponds with observations made in experimental animals.

The relationship of malignant melanoma to UV exposure is much less clear. The incidence data presented by Fears and Scotto show that malignant melanoma of the skin is related to UVR exposure in different ways than nonmelanoma skin cancer. (Malignant melanoma incidence seems to be associated with high brief exposure, rather than cumulative exposure to UVR.)

The evidence *for* the relationship of malignant melanoma to UVR exposure consists of the following: 1) an inverse relationship of malignant melanoma incidence to latitude; 2) relative infrequency in pigmented races; 3) rapid increase in incidence in the past few decades, perhaps due to greater exposure of susceptible populations to UVR; 4) relative preponderance of lesions on the backs of men and on the legs of women; and 5) progression of malignant melanoma from premalignant to invasive lesions, similar to the progression seen in squamous cell carcinoma.

The evidence *against* the relationship of malignant melanoma to UVR exposure is as follows: 1) Malignant melanoma occurs in all animals at sites not exposed to UVR, whereas squamous cell carcinoma occurs spontaneously in animals only at UVR-exposed sites. 2) Only one type of malignant melanoma (the malignant freckle) shows histologic evidence of developing in UVR-damaged skin. By the criteria of anatomic distribution, long latent period, histologic evidence of dermal damage, etc., it is clearly related to UVR. This tumor makes up about 12% of all malignant melanomas and has apparently not increased in incidence in recent decades. 3) Epidemiologic evidence shows no relationship to cumulative UVR exposure. 4) Malignant melanoma other than malignant freckle is a disease of early age.

The best conclusion that can be reached at this time is that in the etiology of malignant melanoma, some major causal effects other than UVR operate, which interact with UV effects. The mechanism seems certainly to be different than that assumed to be operating in nonmelanoma skin cancer.

The final presentations of the conference concerned immunologic parameters of UV carcinogenesis. The elegant work of Kripke and Fisher demonstrated a radically new phenomenon, i.e., that UV pre-irradiation of skin can specifically depress the animal's response to antigenic UVR-induced tumors without suppressing any other immune functions. Detailed studies of this effect show that UVR apparently produces a specific suppressor cell population that interferes with the development of the animal's rejection capacity for UV-induced tumors. The importance of these studies for an understanding of the biology of skin cancers is great. Among other factors, such specific immunosuppression may explain the multiplicity of skin cancers and their onset late in life in man. It suggests that prevention of UVR exposure could reduce nonmelanoma skin cancer incidence by means other than protecting against DNA damage.

In closing, I should like to quote my friend of long-standing, Ian Magnus, who, at the occasion of another conference on UVR effects some years ago, concluded: "The congress was a most happy affair." I found it always stimulating, enormously informative, and an exciting assessment of the state of the art.

On behalf of all participants, I should like to thank Drs. M. G. Hanna, Jr., and Margaret Kripke for their dedicated organizational work, the Misses Harrison, Mowe, Sass, and their staff for the local arrangements, instant typing, and taking care of us so well. The Division of Cancer Cause and Prevention of the National Cancer Institute and the Cancer Biology Program of the Frederick Cancer Research Center are to be congratulated for their vision in sponsoring this conference.

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Recommendations for Future Research on Ultraviolet Radiation Carcinogenesis[1, 2]

Frederick Urbach,[3] John D. Spikes,[4] *and* John H. Epstein[5]

ABSTRACT—Recommendations for future research on photocarcinogenesis were developed during an international conference on UVR carcinogenesis. Areas covered in the recommendations include research at the molecular biology level, human (especially epidemiologic aspects), physical (including instrumentation), and biologic studies (stressing animal models). The recommendations also propose the establishment of central supply and service sources for photocarcinogenesis research as well as the establishment of interdisciplinary training programs related to research in photocarcinogenesis.—Natl Cancer Inst Monogr 50: 203–205, 1978.

In April 1962, an International Conference on the Biology of Cutaneous Cancer was held in Philadelphia. The published proceedings of the conference (*1*) included a number of resolutions and recommendations relating to future research; many of these specifically concerned UVR carcinogenesis (*see* Appendix I). Most of these have not been efficiently implemented and are still highly pertinent, even though styles of research have changed considerably during the past 15 years (e.g., the use of fluorescent sunlamps rather than quartz mercury lamps as UV sources, the use of the dorsal skin of hairless mice as a test object instead of the ears of haired animals, and the recognition of the importance of immunologic considerations in UVR carcinogenesis as described at this conference, etc.).

Interest in UV carcinogenesis has increased sharply in the last few years due to concern over the possible depletion in stratospheric ozone with a resulting increase in the intensity of UV-B radiation at the earth's surface. Since any attempt to evaluate effects of increased UV irradiation on man must be a long-term study, it was believed that an attempt should be made at this conference to suggest what appeared to be the most profitable points of departure for future research on UVR carcinogenesis in humans and in model systems. These recommendations for future research, incomplete as they must be, may provide a useful outline for the organization and funding of research on UVR carcinogenesis.

RECOMMENDATIONS FOR FUTURE RESEARCH

Human Studies

A. Studies on the epidemiology of cutaneous cancers in humans (including genetic and physical characteristics of patients) should be expanded. Such studies should concentrate on the more northern and southern latitudes (greater than 50° and lower than 30°) and on geographical areas where good capabilities for such studies exist (e.g., Australia and Sweden). Such surveys should be repeated in 5–10 years.

B. Attempts should be made to identify high-risk segments of the population and to develop better predictability tests of sensitivity to UVR carcinogenesis, possibly based on persistence of UV-induced erythema and capability for tanning.

C. Immediate efforts to improve and expand environmental UV-B measurements should be coordinated. In particular, personal dosimeters should be used to obtain better estimates of actual human exposures.

D. Better methods need to be devised for estimating cumulative UVR damage to human skin.

E. Improved sun screens, particularly systemic sun screens, should be developed.

Physical Studies

A. Simple, inexpensive personal dosimeters with a spectral response closely approximating the suggested erythema–carcinogenesis action spectrum should be developed.

B. Improved instruments for UV-B measurement in the field should be devised.

C. Well-characterized, standardized UV-B, UV-A, and solar simulator radiation sources for laboratory use should be developed. Simpler and more accurate radiation measuring devices (spectroradiometers) need to be designed and produced for use with these radiation sources.

D. A central quality control facility should be established in the United States for the calibration and standardization of light sources and measurement instruments used in UVR carcinogenesis research; international collaboration in this area is essential.

E. Improved measurements should be made of the optical properties (especially in the UV-B range) of heterogeneous biologic systems (e.g., skin, tissue cultures, etc.) to permit the more accurate determination of absorbed UV dosages.

F. A careful study of the possible uses of UV lasers in research related to UVR carcinogenesis is essential.

Abbreviations: UVR = ultraviolet radiation; UV-B = UVR of 280–320 nm; UV-A = UVR of 320–400 nm.

[1] Presented at the International Conference on Ultraviolet Carcinogenesis held in Warrenton, Virginia, March 21–23, 1977.

[2] These recommendations, developed during the course of the Conference, were discussed at a final Plenary Session.

[3] Center for Photobiology, Skin and Cancer Hospital, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140.

[4] Department of Biology, University of Utah, Salt Lake City, Utah 84112.

[5] Department of Dermatology, University of California Medical Center, San Francisco, California 94122.

Molecular Biology Studies

A. More fundamental work should be done on the UV photochemistry of DNA, especially with wavelengths greater than 290 nm. Particularly recommended is that the types of lesions produced in DNA by wavelengths greater than 290 nm be categorized.

B. A detailed examination should be conducted on possible synergistic or antagonistic effects of different wavelengths of UVR at the molecular–cellular level as well as possible interactions of UVR and chemicals.

C. Additional UVR-sensitive mutant lines of human cells should be developed and their response to UVR analyzed in detail; studies should be done with synchronized cells.

D. The possible presence of error-prone repair and of photorepair enzymes in mammalian cell lines needs to be investigated.

Biologic Studies

A. More detailed research of photocarcinogenesis in experimental animals should be conducted to extrapolate better the results from these simpler systems to the human. In particular, dose–response and reciprocity relationships should be examined in greater detail, and precise determinations of action and promotion spectra for photocarcinogenesis are necessary. Also, the effects of environmental factors (including heat, humidity, wind, etc.) on experimental photocarcinogenesis should be extensively studied.

B. Extensive research should be done to establish relationships between in vivo tumor induction and the molecular events in photocarcinogenesis, photosensitized carcinogenesis, and chemically enhanced photocarcinogenesis (as with promoters and co-carcinogens).

C. In vivo UV damage and repair of DNA in experimental animals need to be compared with the events observed in bacterial and tissue culture systems.

D. One of the most exciting recent developments in animal photocarcinogenesis has been the demonstration of immunologic interactions in the process; this general area of work needs expansion.

E. More research is essential on the development of experimental animal models for malignant melanomas.

ESTABLISHMENT OF CENTRAL SUPPLY AND SERVICE SUPPORT SOURCES FOR PHOTOCARCINOGENESIS RESEARCH

A. Research on experimental photocarcinogenesis would be much more efficient if a central source of animals of known genetic constitution, biologic responsiveness, etc., were established and maintained.

B. Similarly, as described in the "Physical Studies" section, the establishment of a central source of well-characterized light sources and light measuring equipment would enormously improve the intercomparison of research results in photocarcinogenesis as performed in the different laboratories.

C. Finally, a central source for chemical and biologic agents used in photocarcinogenesis research would be useful.

ESTABLISHMENT OF INTERDISCIPLINARY TRAINING PROGRAMS RELATING TO RESEARCH IN PHOTOCARCINOGENESIS

It is apparent from the above listing that research in photocarcinogenesis involves the interaction of an incredibly broad range of specialties. In general, no single laboratory or research institute as presently constituted can hope to provide the necessary broad range of training. Thus it would be useful to establish some kind of interdisciplinary training program involving the cooperative interaction of several photocarcinogenesis research centers.

APPENDIX I

This appendix contains selected, pertinent recommendations from (*1*).

Geographic Pathology and Epidemiology

An attempt should be made to collect from the populations-at-risk as many pertinent anthropologic data as possible, including skin pigmentation, hair and eye color, texture and thickness of skin, and distribution of hair follicles, sweat glands, and pigmented spots according to standard anthropologic and pathologic techniques.

Similar data should be given on sociological, dietary, and cultural habits, including occupation and known or suspected occupational hazards. Clothing and exposure of skin areas and the use of drugs and other similar substances for medicinal and other purposes should also be described.

Geophysical and meteorological data should include altitude, latitude, hours of sunlight, average day temperature, its intensity, rainfall, and humidity, and where practicable, the intensity of UVR.

Etiology

The development of standardized methods of measurement of solar UVR should be encouraged. The coordination and support of meteorologists should be obtained in the study of solar energy and ozone content of the atmosphere.

It is essential to study the differences in the histology and biochemistry of the skin of racial groups and groups of individuals with different degrees of pigmentation, with particular reference to keratinization and melanogenesis, and the effects produced on these and other constituents of the skin by UVR. Groups deserving special attention are persons with albinism, vitiligo, and xeroderma pigmentosum.

Since it will not be possible to eliminate from the environment all agents known or suspected to be carcinogenic, it obviously would be of great value to intensify the search for anticarcinogenic agents.

Pathogenesis

More information is needed regarding similarities and differences between human skin and skin of animal species used in experiments. We need to know more about differences in human skin and what differences occur in the skin of various human ethnic groups. This applies to morphology, chemistry, vascularity, etc. The use of monkeys for experiments is suggested in countries where they can be maintained.

The interaction of carcinogenic agents (chemical, physical, biologic) should be studied in animals, including primates and organ cultures, with particular reference to the effects on the epidermis, stroma, and blood vessels, and at the subcellular level. Attention should be given to terminology of the different agents and their specific action in the neoplastic process.

More consideration should be given to prophylaxis against UVR cancers by the development of protective compounds.

REFERENCE

(*1*) URBACH F (ed): The first international conference on the biology of cutaneous cancer. Natl Cancer Inst Monogr 10: 657–660,1963

APPENDIX

Conference on Sunlight and Skin Cancer

Warrenton, Virginia

March 21–26, 1964

Editors:

Harold F. Blum

Frederick Urbach

INTRODUCTION

On March 21 to 26, 1964, a conference was held at Airlie House, Warrenton, Virginia, under the auspices of the National Cancer Institute, to discuss the etiologic role of sunlight in skin cancer of man. Although much evidence converges to implicate sunlight as a cause of skin cancer, careful examination of this evidence reveals that 1) it is not as complete nor as trustworthy in all respects as might be desired, and 2) some commonly accepted notions are not convincingly supported. Particular interest focuses on the part that sunlight may play in determining the geographical distribution of cutaneous cancer; here the picture is far from simple. This was recognized at the First International Congress on the Biology of Skin Cancer, held in Philadelphia in 1962 (*1*), at which lack of a common basis of comparison for incidence studies and inadequate assessment of the sunlight factor made it difficult to draw valid conclusions.

Assessment of the role of sunlight in this context would demand that we know what portion of sunlight may cause skin cancer and how this particular portion is distributed over the surface of the earth. This is not in itself a simple matter. Granted that if these factors were accurately measured, correspondingly accurate measurements of the incidence of skin cancer among various areas and peoples of the earth would be needed, and the obtainment of these may be complicated by numerous factors: 1) Genetic differences in the skin of different races and the way in which these may affect susceptibility to skin cancer pose numerous questions. 2) Differences in types of skin cancer, their topographical distribution on the body, and the extent to which this may be influenced by sunlight present problems of classification. 3) The obtainment and proper evaluation of incidence data that would be useful in comparative study for different parts of the world offer difficulties, particularly since prolonged exposure is involved.

The nature of some of these problems may explain the constitution of the membership of the conference. Representation is widely spread, both geographically and with regard to scientific discipline (table 1).

The Conference might have been extended to include a much larger group of interested investigators but was limited to facilitate intimate informal discussion, which encouraged recognition and better understanding of problems that need different techniques and points of view for their exploration. As a consequence of this informality, discussion often changed quickly from one topic to another, and an account that followed strictly the order of presentation might be confusing to a reader who was not

TABLE 1.—*Scientists, their professional fields and affiliation*

Scientist	Discipline	Affiliation
Harold F. Blum	Physiology	National Cancer Institute (Bethesda)
Farrington Daniels, Jr.	Dermatology	Cornell University Medical College (New York)
George H. Findlay	Dermatology	University of Pretoria (Pretoria, S. Africa)
Ralph E. Johnson	Radiobiology	National Cancer Institute (Bethesda)
Raymond Latarjet	Biology	Institut du Radium (Paris)
Abraham Lilienfeld	Epidemiology, Biostatistics	The Johns Hopkins University, School of Hygiene and Public Health (Baltimore)
Ian A. Magnus	Dermatology	Institute of Dermatology (London)
Don F. Robertson	Physics	University of Queensland (Brisbane)
Rudolf Schulze	Meteorology (Weather)	Meterologisches Observatorium (Hamburg)
Harold Silverstone	Social and Preventive Medicine	University of Queensland Medical School (Brisbane)
Gerd K. Steigleder	Dermal Pathology	Universitäts-Hautklinik (Cologne)
R. Douglas Sweet	Dermatology	United Hospital (Plymouth, England)
Frederick Urbach	Photobiology	Skin and Cancer Hospital (Philadelphia)
Eugene Van Scott	Dermatology	National Cancer Institute (Bethesda)

present. Hence the following report has been arranged by the editors in what seems a logical order of subject matter, without close regard to the sequence in which the remarks of the various participants were made. Most of the following account was prepared by the editors from notes taken at the meeting but was submitted to the discussants for correction and revision.

Harold F. Blum
Frederick Urbach

PART I

GENERAL HISTORY AND OUTLINE OF THE CONCEPT

Harold F. Blum

Although the earliest association of skin cancer with sunlight is commonly credited to P. G. Unna (*2*), the first important attempt at epidemiologic study was made by William Dubreuilh around the turn of the century (*3*). Dubreuilh found that the incidence of skin cancer among inhabitants of the city of Bordeaux was lower than among workers in the neighboring vineyards. He also noted that these cancers appeared chiefly on the face or other exposed parts. As a result of these and other early studies (*4*), the idea that sunlight is a causal factor in human cutaneous cancer became generally accepted, at least among dermatologists. At this stage, the evidence supporting this concept might have been outlined as follows:

1) Skin cancer appears in greatest amount on the exposed parts of the body [this is a common observation (*5*)].

2) Negroes, who sunburn less readily than white-skinned people, have less skin cancer [this is also clearly demonstrated (*6*)].

3) Among white-skinned people, outdoor workers have more skin cancer than do those who work indoors. This is difficult to demonstrate, and all studies are not in agreement.

4) More skin cancer occurs among white-skinned people living in areas where insolation is greater. Since the early workers did not know what portion of sunlight might be involved nor its distribution over the earth's surface, their conclusions in this regard cannot be taken seriously; indeed, this part of the evidence is still incomplete as will be apparent from the following discussions.

The argument could not be developed much further at the time, and research remained static for a decade or two until interest revived after the experimental studies of G. M. Findlay in 1928, who, in exploring the effects of mercury arc radiation on carcinogenesis by tar, found that this radiation alone would induce cancers in the skin of mice and rats (*7*). Within a few years, similar findings were made independently by Putschar and Holz in Göttingen (*8*); Herlitz, Jundell, and Wahlgren in Stockholm (*9*); and Roffo in Buenos Aires who also produced skin cancers in rats by exposure to natural sunlight (*10, 11*).

The part of the spectrum that produces cancers was defined by Funding et al. (*12*) prior to 1936. By using spectrally calibrated filters and large numbers of mice, they found that only wavelengths shorter than about 320 nm were carcinogenic.[1] In 1933, Roffo had made similar studies with rats (*11*), but his filters were only designated by color. However, we may reasonably assume that Roffo's "verre transparent" which, in his experiments on rats, set the long wavelength limit, was ordinary window glass that cuts wavelengths shorter than about 320 nm. Later, Rusch, Kline, and Baumann (*13*) obtained results corresponding to those of Funding and his co-workers. An unfortunate mistake in interpreting a published account led some to believe for a time that longer wavelengths in the UV may induce cancer (*14*); however, it seems that the limit of about 320 nm may be accepted for mice and rats (and by inference from other relationships that will be mentioned) to hold also for carcinogenesis in man.

Text-figure 1 shows the general spectral constitution of sunlight reaching the earth's surface. Even at maximum conditions, no wavelengths are shorter than about 290 nm; since the carcinogenic wavelengths end around 320 nm, they constitute only a small fraction of the whole (*see* text-fig. 2). The distribution with latitude, season, and time of day for this small UV fraction is different from that of total sunlight or of the portion to which the human eye responds, and measurement of the former constitutes a special problem, as will become clear in subsequent discussion.

Many other effects of UV light are produced only by wavelengths shorter than 320 nm including: lethal and genetic effects on microorganisms, delay of cell division, lysis, aberrations in development, and, of particular interest to us, *sunburn* in man. Nucleic acids absorb only wavelengths shorter than about 320 nm, and these are clearly the light absorbers or chromophores for genetic and lethal effects and probably others. Unconjugated proteins also cease to absorb at this long-wavelength limit and are likely chromophores for some effects. Other biologically important substances are possibly concerned sometimes. It may be noted that antirachitic action, which involves a steroid compound, also has its long-wavelength limit here.

Whatever the relationship between carcinogenesis and sunburn, their common wavelength range associates them, and it seems likely that some basic similarity exists in their mechanisms, although this is not established. Several investigators have studied the action spectrum for the erythema of sunburn, and a standard "erythemal curve," shown in text-figure 2 (CIE), was adopted by the Comité

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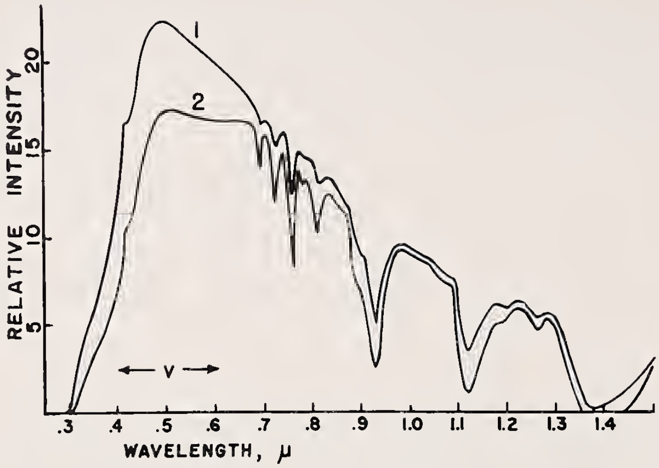
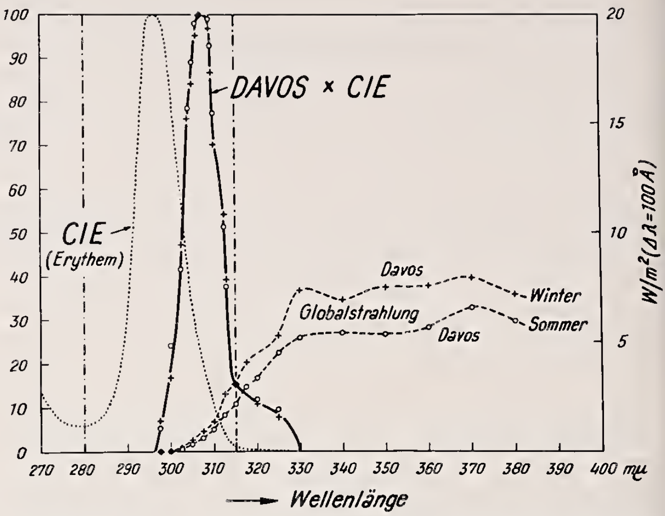
[1] Although wavelengths were given in "mμ" at the time of this conference (1964), they have been edited to read "nm" throughout this Appendix to conform to the current style of the *Journal of the National Cancer Institute.*



TEXT-FIGURE 1.—Relative spectral distribution of the energy of sunlight at the earth's surface: 1, with the sun at zenith (solar angle 90°); and 2, at 60°, 4 hr from zenith (solar angle 30°). V indicates the approximate limits of human photopic vision. Absorption by ozone in the earth's atmosphere sets the short wavelength (UV) limit at about 0.29 μ (290 nm). Indentations in the IR spectrum are principally due to absorption by water vapor [from Moon *(15)*].



TEXT-FIGURE 2.—UV spectrum of sunlight and spectrum of erythemal action. *Broken lines* indicate the total radiation (direct and sky), estimated for winter *(crosses)* and summer *(circles)* at Davos, Switzerland [based on Bener's *(16)* measurements]. CIE = standard erythemal curve. Spectral range called UV-B is indicated at the *top* of the diagram. Davos × CIE = curve obtained by multiplying the values for sunlight by the corresponding values for the erythema spectrum. *Left hand ordinates:* relative values for CIE and Davos × CIE, with maxima at 100. *Right hand ordinates:* watts per square meter per 100 Å (10 nm).

International de la Lumière[2] at its Third International Congress in 1936.

Such a curve measures only one aspect of sunburn and varies according to the way in which the measurements are made and from person to person; thus it should be considered only as an approximation to the action spectrum of sunburn. Lacking an action spectrum for carcinogenesis (which has not yet been determined), the CIE curve may be used as an approximation, but if this is done, it should be remembered that we are introducing another approximation and may be compounding errors. No other approach seems reasonable; however, some latitude must be allowed in estimates of the "carcinogenic" UV of sunlight.

Dubreuilh and the other early dermatologists who developed the idea that sunlight causes skin cancer were, of course, altogether unaware of the problems concerned in the measurement (which had not yet been attempted) of sunlight and of action spectra. They probably had no clear idea of what needed to be measured, and it is a tribute to their intuition that despite these limitations, they were able to carry the concept as far as they did. Our knowledge has greatly increased since then in other pertinent fields, as will become obvious in subsequent pages. Today we may approach the problem much more critically. In so doing, we are sure to find discrepancies and inadequacies of both data and point of view that may affect the status of the whole concept.

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[2] Since 1951, this group is called the Comité International de Photobiologie.

PART II

MEASUREMENT OF SUNLIGHT: THE ULTRAVIOLET "B" RADIATION IN SUNLIGHT

Rudolf Schulze

Warrenton, Virginia, where this meeting is being held, has nearly the same latitude as Sydney, Australia, and one might assume that, other things being equal, the incidence of ultraviolet radiation (UVR) would be about the same. Yet a high incidence of skin cancer is reported from Australia and apparently little from Virginia. This would seem to illustrate one kind of problem this conference is considering.

In an attempt to measure the incidence of solar radiation, no matter what the spectral region concerned, one must take into account both the direct radiation from the sun and that scattered from the sky, i.e., total effective radiation is the sum of direct and scattered light. Direct and scattered radiation are functions of the angle the sun makes with the earth's surface. We speak here of the angle from the horizontal as the *solar angle.* This angle varies with season of the year, latitude, and hour of the day. The direct and the scattered light vary with solar angle but not to the same extent for all wavelengths. The scattering from the sky increases greatly as wavelength decreases, so the ratio of scattered to direct radiation is much greater in the UV than in the visible. Thus our eye gives us no measure of the radiation that causes sunburn (cf. text-figs. 1, 2).

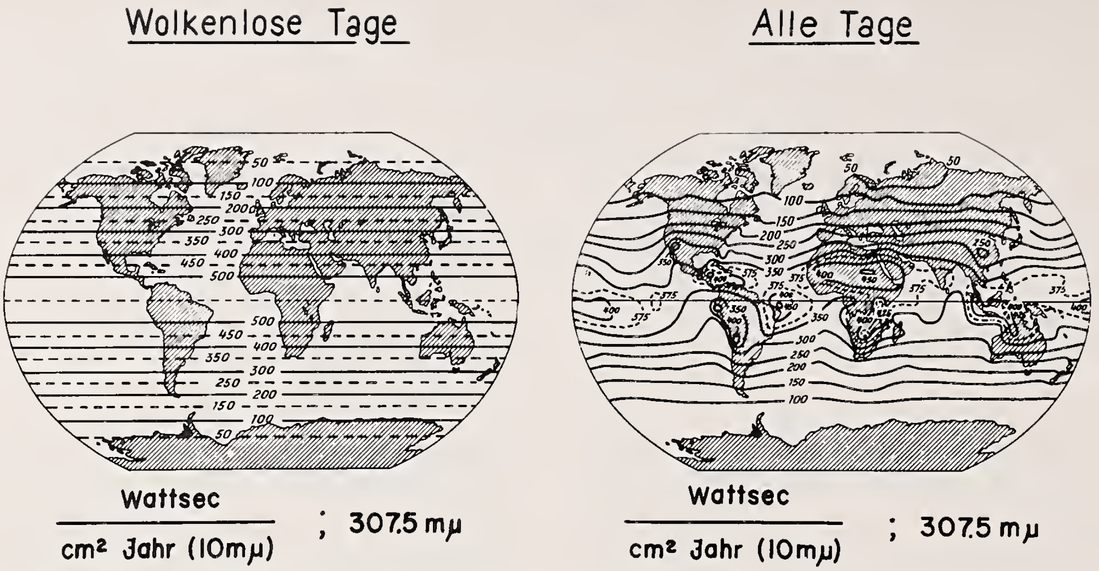
The sunburning radiation in sunlight, which lies between 290 and 330 nm, will be designated "ultraviolet B" (UV-B), following the suggestion of Coblentz and adopted by the Comité International de la Lumière[2] in 1936; the longer UV wavelengths are designated "ultraviolet-A" (UV-A), and the shorter ones beyond the limit of sunlight "ultraviolet-C" (UV-C). Text-figure 2 shows the distribution of the total UVR of sunlight, both direct and that reflected from the sky and the terrain, as measured for winter and summer at Davos, Switzerland by Bener (*16*); also shown is the CIE erythemal curve.

To obtain estimates of the erythemal effectiveness of sunlight, one may multiply values from the CIE curve by the energy values for sunlight at corresponding wavelengths; the curve labeled Davos × CIE in the text-figure has been obtained in this way. If corrected to the same common maximum value as in the figure, the curves for winter and summer sunlight are similar in shape. The maximum of this effectiveness curve is at 307.5 nm, well toward longer wavelengths from that indicated by the CIE curve, which is at about 297 nm. The long-wavelength limit shifts to about 330 nm. As an index of the relative erythemal effectiveness under different conditions, the 307.5 nm wavelength has been used to make various estimates, all of which are based on Bener's measurements.

Such estimates of the world distribution of UV-B per annum, are presented in text-figure 3 and are given in watt seconds/cm^2 × 10 nm. On the left of the figure is a map of the distribution of UV-B based on the assumption of cloudless days throughout the year. On the right is a map of UV-B corrected in terms of the annual cloudiness, which indicates that another important factor is determining the carcinogenic dosage from sunlight. Such a curve is much different from that which would be obtained for corresponding measurements of visible or total sunlight or even for the longer UV. Text-figures 4 and 5 show the world distribution for the months of July and January, respectively. These figures indicate that the UV-B is much less concentrated in the tropics than has often been assumed; the high amounts in the arctic regions may seem surprising at first, but sunburn is not uncommon there (text-fig. 6). The UV-B is the same in Australia and Virginia.

The variation of total UVR with solar angle is estimated in table 2 for high mountains and sea level.

We performed some experiments on a group of human subjects who were exposed to sunlight at three altitudes (*18*). At sea level in Berlin, the altitude is 150 m, and at the Jungfrau Loch, it is 4,000 m. Exposure was made at the same solar angle in all three places on cloudless days. The end point was always nearly the same degree of erythema. The length of the exposure required to reach this degree of erythema was taken as a measure of the dosage applied. In Berlin, 10% more exposure was required than was estimated from the calculated curve in text-figure 2, whereas at the Jungfrau Loch, it took 20% less than the estimate. The differences are presumably due to differences in scattering of the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Wattsec / cm² Jahr (10mμ) ; 307.5 mμ
Wattsec / cm² Jahr (10mμ) ; 307.5 mμ
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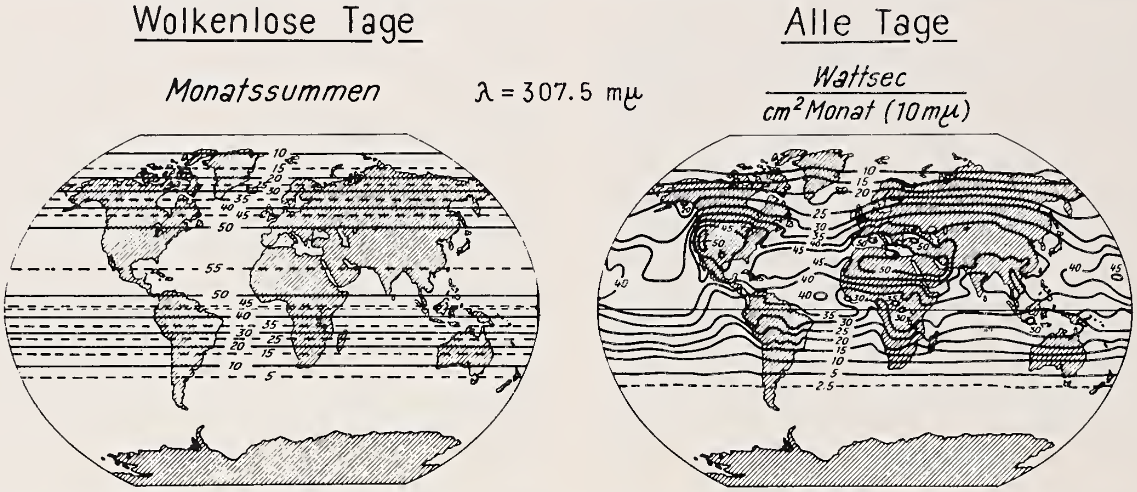
Text-figure 3.—World distribution of UV at 307.5 nm per year, in watt seconds per square centimeter (10 nm). *Left map* is estimation for cloudless days throughout the year. *Right map* is estimation for total days.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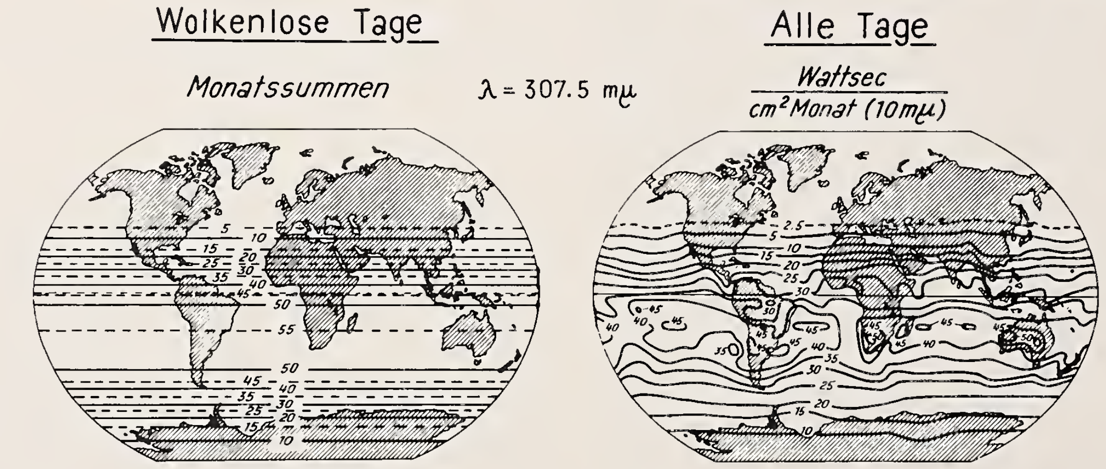
Text-figure 4.—World distribution of UV at 307.5 nm for the month of July in watt seconds per square centimeter (10 nm). *Left map* is estimation for cloudless days throughout the month. *Right* map is estimation for total days.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Text-figure 5.—World distribution of UV at 307.5 nm for the month of January, in watt seconds per square centimeter (10 nm). *Left map:* cloudless days throughout the month. *Right map:* total days.

TABLE 2.—*Variation of total UV-β with solar angle*[a]

Solar angle, degrees	High mountains[b] Summer	High mountains[b] Winter	Sea level[c] Summer	Sea level[c] Winter
0	1.80×10^{-4}	3.50×10^{-4}	1.3×10^{-4}	2.4×10^{-4}
5	7.60×10^{-4}	1.32×10^{-3}	5.3×10^{-4}	9.2×10^{-4}
10	2.24×10^{-3}	4.45×10^{-3}	1.57×10^{-3}	3.1×10^{-3}
15	7.60×10^{-3}	1.31×10^{-2}	5.3×10^{-3}	9.2×10^{-3}
20	1.91×10^{-2}	3.25×10^{-2}	1.34×10^{-2}	2.3×10^{-2}
25	4.05×10^{-2}	7.08×10^{-2}	2.8×10^{-2}	5.0×10^{-2}
30	8.00×10^{-2}	1.30×10^{-1}	5.6×10^{-2}	9.1×10^{-2}
35	1.50×10^{-1}	2.13×10^{-1}	1.05×10^{-1}	1.49×10^{-1}
40	2.60×10^{-1}	3.29×10^{-1}	1.82×10^{-1}	2.3×10^{-1}
45	3.85×10^{-1}	4.70×10^{-1}	2.7×10^{-1}	3.3×10^{-1}
50	4.90×10^{-1}	6.30×10^{-1}	3.4×10^{-1}	4.4×10^{-1}
55	5.70×10^{-1}	7.85×10^{-1}	4.0×10^{-1}	5.5×10^{-1}
60	6.30×10^{-1}	9.40×10^{-1}	4.4×10^{-1}	6.6×10^{-1}
65	6.65×10^{-1}	1.09	4.7×10^{-1}	7.6×10^{-1}
(75)[d]	(8.6×10^{-1})	(1.13)	(6.0×10^{-1})	(7.9×10^{-1})
(90)[d]	(9.7×10^{-1})	(1.25)	(6.8×10^{-1})	(8.8×10^{-1})

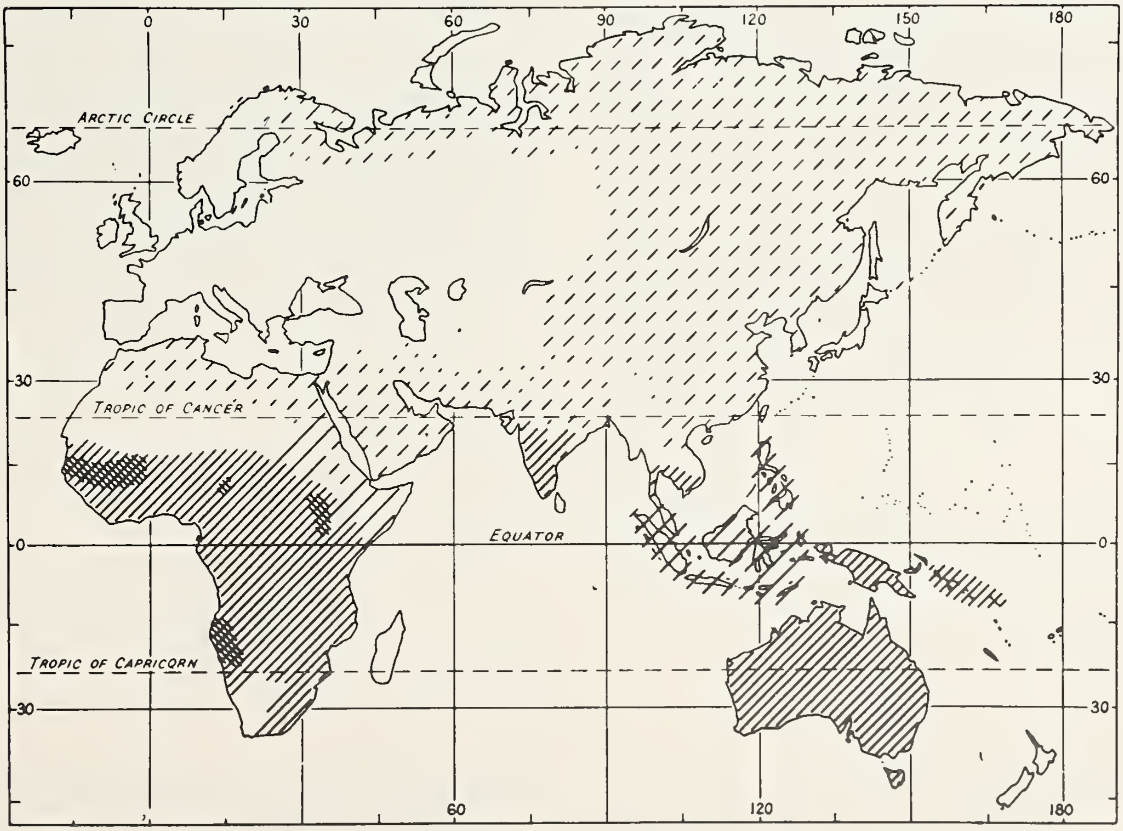
[a] Values are expressed as watts per square meter at 307.5 nm.

[b] Data are from Bener *(16)*, at Davos (altitude, 1,590 m).

[c] Values were estimated as 70% of the high mountain value.

[d] *Values in parentheses* were obtained by graphical extrapolation.

light by airborne particles at the lower altitudes. This finding would seem to disperse the accepted myth of "biologic darkness" in cities. (However, in our discussion, some stated that there might be less absorption by the aerosols over Berlin than over smog-ridden cities such as London or Los Angeles.)



TEXT-FIGURE 6.—Distribution of skin color in the eastern hemisphere. *See* page 241 for detailed discussion. [Reproduced with the permission of *Geographical Reviews* (*17*).]

MEASUREMENT OF CARCINOGENIC ULTRAVIOLET IN SUNLIGHT

Don F. Robertson

Many factors are known to have some bearing on the development of skin cancer. In a quantitative assessment of the relative importance of these, a study of the UV spectrum of sunlight is essential. Ample evidence proves that only the shortest wavelengths of sunlight have a marked carcinogenic action in skin. Thus the carcinogenic part of sunlight carries a small fraction of the total energy in sunlight and is affected most sensitively by physical factors such as elevation of the sun and the state of the atmosphere. Skylight differs from direct sunlight and both vary in spectrum and relative intensity with position on the earth's surface and time of the year. Since skin cancer is normally the consequence of many years of exposure, we must determine the integrated exposure over long periods as well as the variations in spectrum and intensity. It is particularly desirable that comparable estimates of exposure be made for different localities where cancer incidence studies are being made.

Once a reasonable action spectrum for the development of skin cancer has been accepted, two approaches are needed in a comparison of carcinogenic exposures at different parts of the earth's surface.

Spectral Analysis

Schulze combined Bener's analysis of the measured spectra of sunlight and skylight on cloudless days with the erythemal action spectrum to show how the erythemal effectiveness varies with latitude and time of the year (text-figs. 3–5). From this, yearly total exposures at different places may be compared, provided that different atmospheric conditions do not interfere with the comparison. This is valuable information. However, climatic differences over the land masses do make a systematic difference in the shortest UV wavelengths penetrating to the earth's surface. Common cloudy skies cause rapid changes of intensity and spectral distribution that do not lend themselves to combination with a postulated action spectrum for calculation of relative effectiveness. The effect of cloud, dust, haze, smoke is unpredictable and well worth examining by direct measurement.

Integration of All Active Wavelengths

Continuous measurement of the appropriate band of UV rays is the simplest way to take into account the influence of local climate. Ideally, we could use an instrument that has a spectral response that varies in exactly the same way as the carcinogenic action spectrum. This would permit comparison of the average effectiveness of all the radiation from sun and sky. Continuous integration of exposure is required that gives daily or monthly totals with some record of the intensity averaged for about 1-hour intervals. Since the places offering useful comparisons of occurrence of skin cancer in the population are generally not those where well-equipped solar observatories are established, this equipment should preferably operate reliably for long periods without interruption or skilled attention, and it should be simple and semiautomatic. From a long series of such measurements, differences between exposures at various localities may be compared with those anticipated from Schulze's analysis and with differences in the carefully recorded incidence of skin cancer in the population. Any lack of correlation between cancer incidence and measured UV would suggest that either we were not measuring the most effective wavelengths or other important factors, which might then be recognized more positively, were present.

Since no action spectrum for carcinogenesis in skin has been accurately established, it appears acceptable to design an instrument with spectral sensitivity that will reproduce the standard erythemal action spectrum. Then if this instrument can be realized, even approximately, it is essential that its spectral response remain constant long enough to permit useful comparison, i.e., for some years. A number of such instruments are required, all with the same shape of spectral response. Calibration of the instrument will include the spectral characteristic and will not be in absolute physical units (unless expressed as for a nominal mean wavelength e.g., 297 nm). Although there is no shortage of methods for measuring the quantity of UVR, few have the spectral shape and constancy required. Many investigators have measured sunlight in their locality with their particular type of instrument. It is impossible to combine or compare these, if the detectors have neither been equipped with similar spectral sensitivities nor have been calibrated by comparable standard sources.

It is surprising how many workers who use selective detectors of UVR have merely quoted the sensitivity in terms of microwatts. For any reasonable comparison of results from different places, it is essential that full details of the calibrating technique be given.

Choice of Detector

The instrument required will be sensitive in the wavelength band of 290–330 nm but must be completely insensitive to the intense visible component of sunlight. It should have its maximum sensitivity about 297–300 nm, fall sharply to about 320 nm, and have negligible response above 330 nm.

Spectrally Selective Detector

Any system that shows a reaction to the energetic photons beyond a threshold at a 320- to 330-nm wavelength must be considered.

Vacuum photocells with cathodes such as titanium, zirconium, and magnesium have been used by many workers. They have the advantage of a sharp threshold but do not rise rapidly in sensitivity to a maximum about 300 nm. It is not easy to obtain a number of cells with the same threshold and the same spectral response. Sudden changes in response after prolonged use have been reported.

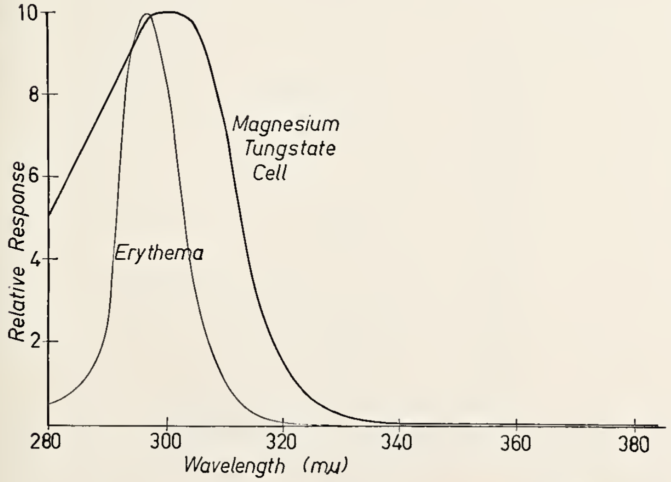
Some chemical reactions are sufficiently close to the required spectral response to be useful for special applications. They do not lend themselves to continuous recording without frequent attention.

Fluorescence of some phosphors commences sharply between 320 and 330 nm and rises rapidly in sensitivity down to 297–300 nm. Magnesium tungstate seems the most suitable and has been incorporated into a satisfactory continuous recorder in our laboratory in Brisbane.

Spectrally Selective Filters

In general, filters do not cut off as sharply or rise as rapidly in transmission as we require. A suitable response curve may be obtained by taking the difference in transmission between two filters with different cut-off points; however, this does not provide a simple continuous integration of exposure. It also uses the small difference between two large quantities in which minor errors would be troublesome.

Interference filters decrease rapidly in transmission from a sharp maximum and can be made to match the upper part of the erythemal action spectrum. However, transmission at longer wavelengths does not fall low enough for an interference filter to be of any use in sunlight without a supplementary filter. Since the peak transmission varies with angle of incidence, an interference filter is not suitable for global radiation from the sky. Christiansen filters have a similar spectral transmission but are sensitive to temperature.



TEXT-FIGURE 7.—Spectral sensitivity of magnesium tungstate cell in relation to erythemal sensitivity.

Any filter system which must absorb all of sunlight except the small erythemal fraction will run hot in summer. The effect of this temperature rise on the filter or on the detector below it must be considered.

The wide range of detectors that may be used to measure the selected radiation penetrating the filter may be divided into two broad groups: those with response uniformly proportional to intensity for all wavelengths and those with nonuniform spectral response.

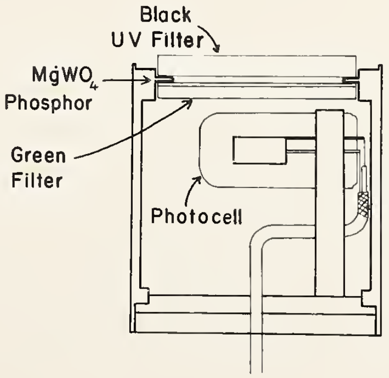
Uniform detectors, such as thermopiles and bolometers, generally need sensitive electrical recorders. A new chemical detector with fairly uniform spectral response and useful sensitivity uses potassium ferrioxalate (*19*).

When nonuniform detectors are used with filters, the overall spectral response depends on the combination. In some instances, the detector provides the critical threshold, in others it does not. Detectors in this class include photoelectric, photovoltaic, and photoconductive cells; photomultipliers; TV camera tubes; some phosphors; and some photochemical reactions. Photographic materials are generally fully sensitive about 300 nm but normally rely on acceptance of a wide range of wavelengths to operate at the high speeds of normal photographic work. When only a narrow fraction of the spectrum is used, all photographic emulsions appear much slower.

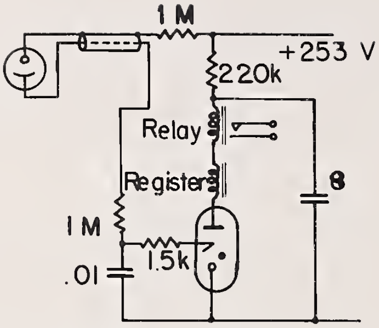
Magnesium Tungstate Detector

Equipment developed in our Physics Laboratory and improved in the field over 7 years has proved reliable for comparison of exposure at different places. The response spectrum is reasonably close to the erythemal action spectrum (text-fig 7). Units with the same spectral response can be produced and the sensitivity remains constant over long periods. The electrical recording system is simple and reliable.

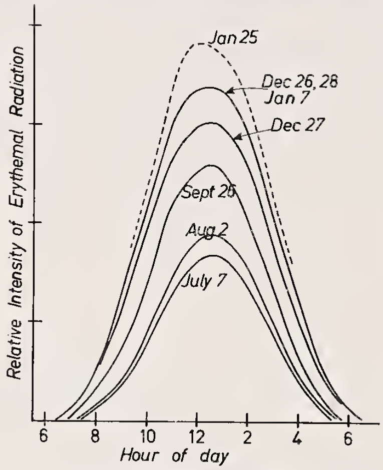
A layer of magnesium tungstate phosphor, used as the primary detector (text-fig. 8), has a threshold about 330 nm that rises rapidly to a maximum at 300 nm, below which it falls. Fluorescent emission by the phosphor has its maximum in the green part of the spectrum and is measured by a vacuum photocell behind a narrow-band green filter (Corning 4010). Direct action of the sunlight on the photocell is prevented by placement (immediately above the phosphor) of



TEXT-FIGURE 8.—Vertical cross section of magnesium tungstate detector. The flat silica cover plate is omitted from the diagram.



TEXT-FIGURE 9.—Diagram of integrating-counting circuit.



TEXT-FIGURE 10.—Variation of erythemal intensity during cloudless days at Townsville at different times (1963–1964) measured with a magnesium tungstate detector. January 25 was a most exceptional day.

a 6-mm thick rough moulded slab of Corning 9863, which removes the strong visible spectrum most effectively but transmits UV-A and UV-B. (The available 3-mm polished plates of the same glass do not provide sufficient absorption of the intense visible portion of sunlight.) The green filter removes any UVR, red, or IR radiation which penetrates the "black" glass (Corning 9863) filter. The filters and phosphor plate are about 3 inches square. Weathering of the soft 9863 glass can be prevented with a layer of clear silica (Corning 7910).

Current from the photocell (RCA 1 P 39 or Mullard 150 AV) is about 0.01 μA in midsummer and is conveniently integrated (text-fig. 9) by a polystyrene capacitor attached to a trigger tube (Mullard Z803U).

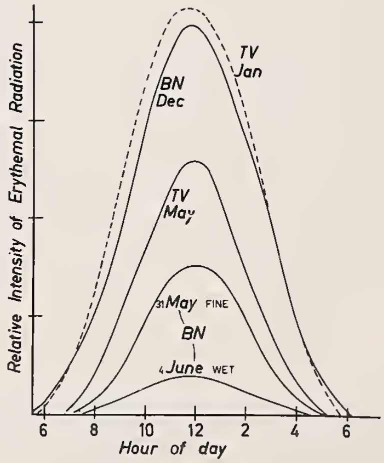
The trigger tube fires at about 135 V, drops the condenser voltage to 90 V, and automatically stops conducting. With the use of a selected stabilized voltage supply, these electrical characteristics remain constant, so that the number of discharge cycles is proportional to the erythemal exposure. Each discharge is tabulated on a simple impulse counter that provides the progressive total and on a printing counter that gives the total in each half-hour.

Equipment of this type has been tested for 4 to 5 years at Cloncurry (dry tropical inland), Townsville (relatively cloudy tropical coastal), and Brisbane (subtropical coastal). Dr. Silverstone will elaborate on these studies in his paper (p 230). Useful comparisons of total exposures (on a horizontal surface) and of variations in exposure have been obtained. The use of flat filter glasses above the phosphor layer causes the sensitivity of the detecting system to decrease for radiation entering at low angles. Because of the spreading of much of the UV-B in the blue sky and the average high angle of the sun, angle of incidence effects do not disturb the comparisons made at these three places. When a wider range of solar elevation is to be compared, hemispherical filters are proposed above the plane of phosphor.

Results

Examples of the information being obtained are shown in text-figure 10 (variation during cloudless days at Townsville at different times of year) and text-figure 11 (the maximum range from Townsville summer to a wet winter day in Brisbane). Text-figure 12 compares integrated monthly totals at the three areas for 4 consecutive years. If either total exposure or intensity of clear days is compared, the difference between radiation levels in the two tropical areas and in subtropical Brisbane is not nearly as striking as the difference for incidence of keratoses and skin cancer shown by Silverstone.

On a clear midwinter day in Brisbane, it is necessary to expose normal skin for the entire day to cause minimal erythema. In midsummer, exposure for about 15 minutes will have the same effect. Yet the midday erythemal intensities only differ by a factor of 4. This suggests a mechanism of tolerance or of recovery operating in the biochemical progress toward erythema. If a similar recovery mechanism reduces the cancerogenetic actions, minor variations in exposure rate will exert a more obvious difference on the rate of tumor development.[3]



TEXT-FIGURE 11.—Diurnal variation of erythemal UV intensity on high and low days at Townsville (TV) and at Brisbane (BN).

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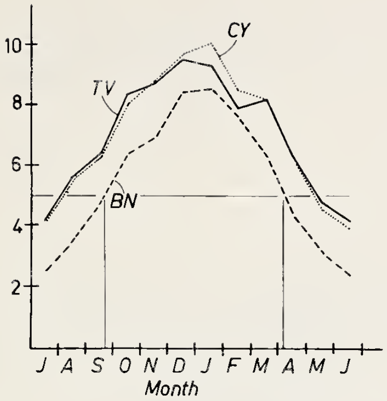
[3] Experimental studies indicate failure of reciprocity at low dose rates of UV light (*20*).



Text-figure 12.—Relative average monthly totals of erythemal UV for 4 consecutive years. TV = Townsville; BN = Brisbane; CY = Cloncurry.

In midsummer, the daily total of erythemal rays in Brisbane is only slightly below that in Townsville or Cloncurry. Even Melbourne is within 15% of the maximum tropical exposure at sea level. However in winter, the tropical monthly totals barely drop below half the midsummer values, whereas Brisbane is below this half-maxmum level for about 5 months of the year. Exposures less than 50% of those at Cloncurry and Townsville do not seem to be associated with much skin cancer since, on anual totals, these are equivalent to those in the south of Canada or of England. If the more effective contribution to carcinogenesis comes from intensities greater than this level, the tropical regions are exposed at active levels for at least 10 months of the year, whereas Brisbane has 7 months at slightly lower intensity. The amount of exposure above the 50% average level is just twice as much at Cloncurry and Townsville as it is in Brisbane. There is a certain degree of coincidence in the fact that Silverstone's second text-figure shows that the rate of rise in skin effects with age is about twice as great in the tropics as it is near Brisbane. Nevertheless, it does seem that comparison of higher intensity exposures for longer periods is more significant than is a simple comparison of annual totals.

HISTORICAL NOTE

Harold F. Blum

In 1941, the National Cancer Institute planned to make measurements of UV-B at various places in the United States; the United States Weather Bureau was to have collaborated in this endeavor. Under the direction of Dr. Frederick Bracket and using Rentschler photoelectric cells with an integration counter, researchers did field studies at two stations in this country. Although this instrument might remain stable in the field for a long time, these studies showed that it was subject to sudden changes in sensitivity (*21*). The cause of the changes was not determined, but it was hoped that this difficulty might be corrected; a number of photocells with special flat receiving surfaces were constructed for the purpose in Dr. Rentschler's laboratory. At this time, the United States entered World War II; those who might have continued this work became involved in war research of one kind or another, so the project was abandoned and forgotten. At the end of the War, I searched for the photocells that had been stored but discovered that all were broken. It is likely that these cells would not have been satisfactory for field work; however, with revived interest and more suitable methods, such a program might again merit consideration.

PART III

TYPES AND DISTRIBUTION OF SKIN CANCER AND ASSOCIATED LESIONS

The various kinds of skin cancer are distributed differently on the body. In the earlier studies this was not taken into account, and the large predominance of all skin cancers on the exposed areas were accepted as proof that sunlight was the principal cause. One may question the role of this agent in the various kinds of skin cancer. For example, the melanomas are frequently found on regions of the body, such as the pubic area and the soles of the feet, where sunlight could have no effect. One may also question the influence of sunlight in the development of this type of cancer when it occurs on exposed areas. The basal cell and squamous cell carcinomas show different distribution on the exposed parts; again, the question is raised about the importance of sunlight in the induction of these two types of epidermal tumor. Other lesions of the skin, nonmalignant in character, are generally attributed to the action of sunlight, e.g., the so-called solar keratoses regarded by many as precancerous, and other changes associated with aging.

The classification of types of lesions is essential in studying their relation to sunlight. If incidence is to be compared at different areas of the earth, the same method of classifying the lesions must be followed. Of course, the same is true for the topographical distribution of lesions.

CLASSIFICATION OF SKIN TUMORS AND ASSOCIATED LESIONS

C. K. Steigleder

Various kinds of tumors and associated lesions of the skin are recognized: precancerous, benign, and malignant. The latter may be defined as tumors that can kill, which seems to be a generally acceptable definition of cancer. A classification of all these lesions is presented in table 3.

Sunlight can have an effect in only some of these types of tumors. The sarcoma group can probably be eliminated because it is unlikely that the UV-B penetrates to the human dermis in sufficient amount to cause carcinogenesis there. Similarly, systemic diseases with cutaneous manifestations, such as Bowen's disease, cannot depend (etiologically) on sunlight but may nevertheless complicate the problem with which we deal.

Interrelationships Between Precancerous and Cancerous Lesions

In some cases, it may be difficult to distinguish definitively between types, and questions arise of interrelationships between precancerous and cancerous lesions. Questions not clearly answerable at present but which may influence the present problem are listed below.

1) Do precancerous lesions of skin exist? How should a precancerous lesion be defined, based on recent advances in tumor pathology and experimental oncology? Is the classical definition still justified that precancerous lesions of the skin are clinically and histologically visible changes of the skin in which cancer will develop in a high percentage?

2) Do precancerous lesions give rise to basaliomas, e.g., what percentage of basalioma will develop in actinic keratosis? Is actinic keratosis different from other keratoses? Do Bowen-like patterns in actinic keratosis indicate internal carcinoma? What criteria will prove that a basalioma has developed in actinic keratosis?

3) Is superficial basalioma related to other basaliomas, since superficial basalioma-like changes are observed in epidermis covering histiocytomas of patients of a certain age group?

4) What criteria justify the diagnosis of a carcinoma of epithelial appendages?

TABLE 3.—*Types of skin tumor*

	Group		
Type	Epithelioma	Melanoma	Sarcoma
Precancerous	Local		Local
	Senile lentigo	Premalignant melanosis	
	Keratoses (actinic, chemical)		
	Erythroplasia		
	Cheilitis		
	Systemic		Systemic
	Bowen's disease?		Parapsoriasis en plaques?
			Neurofibromatosis v. Recklinghausen
Benign	Local		Local
	Superficial basalioma	Superficial melanoma?	Dermatofibrosarcoma protuberans
	Basalioma		
			Hemangiopericytoma
	Systemic		Systemic
			Mycosis fungoides
	Multiple basaliomas		Hodgkin's disease
			Chronic leukoses and related disorders, such as reticuloses and angiosarcomatosis Kaposi
Malignant	Prickle cell carcinoma	Melanoma	Local
			Sarcomas of various types
			Systemic
	Carcinoma of epithelial appendages		Reticulosarcomatoses and related disorders

5) Are there transitions between basaliomas and other carcinomas?

6) Is superficial melanoma different from other melanomas and premalignant melanosis (Hutchinson-Dubreuilh)?

7) Is a melanoma that develops in premalignant melanosis different from other melanomas? How may it be distinguished?

8) Is the separation of superficial melanoma justified as far as the prognosis is concerned?

Changes in the Skin With Age

The skin undergoes characteristic changes with age, and since these are generally more pronounced in exposed areas, sunlight may influence exacerbation of these lesions.

In age, the dermal papillae become flattened, and the dermis becomes thinner; the dermal septa, which separate the subdermal adipose tissue, tend to disappear.

The direction of the collagen bundles in the dermis changes. In a young individual, these bundles appear as a rhomboidal network, whereas in older people, the bundles run predominantly parallel to the flattened epidermis. This arrangement is especially well demonstrated in the X-ray picture of histologic sections called "microradiogram" or "histioradiogram" (*22*). In addition in older individuals, the collagen bundles appear quantitatively different. They are either broad, with low absorption of X-rays or extremely thin with high absorption. In light-exposed areas, the normal collagen is replaced by pathologically altered material which, according to our determination, is neither collagen nor elastic tissue but a connective tissue of intermediate character and low quality. Several methods are available to determine the degree of this elastotic degeneration. It should be possible to establish a quotient between elastotic degeneration of a given light-exposed area and biologic age of skin not exposed to light.

CLINICAL INVESTIGATIONS

R. Douglas Sweet

When incidence of various skin cancers is discussed, it is of utmost importance that the exact histologic diagnosis be ascertained and the exact site of the lesion be recorded for each case. Without this precise knowledge, any survey is useless.

It is also important to distinguish what (for want of a better word) I call the "nevoid type" of skin cancer from the sort induced by external influences such as UV-B, chemical carcinogens, etc. This applies particularly when skin cancer in young people is discussed. Although difficult to ascertain we should agree on type of transition point histologically between keratoses and squamous cell carcinomas.

I am sure it would be useful in years to come if proper reliable and comparable measurements of the actual amount of UV-B were made in different parts of the world. I think Dr. Robertson's apparatus (especially because it is so simple) should be exploited to the full. Meteorologic stations in a number of countries should keep records taken from this type of machine for several years.

DIFFERENCES BETWEEN SQUAMOUS CELL AND BASAL CELL CANCERS

Eugene Van Scott

One of the few known factors concerning the difference between squamous cell and basal cell cancer is that the cells above the basal layer in the former have the ability to divide as well as to keratinize. This may be explained on the basis of selection of clones of cells having increased potential for division (*23*). In contrast, in basal cell carcinoma, cells lose the capability to produce keratin. Evidence for this includes electron micrographs that show basal cell carcinomas have few, if any, malformed tonofilaments (*24*) and results of chemical studies that reveal the tumor population lacks the normal keratinous protein fraction precipitable at pH 5.5 (*25*).

Since the cells in the basal cell carcinoma cannot keratinize, they are unable to migrate outward and are trapped within the skin, but they continue to divide normally. Although the cell of the basal cell carcinoma is otherwise normal, characteristically little or no inflammatory reaction is observed around the tumor, whereas an inflammatory reaction characteristically occurs with squamous cell cancers. Both types of cancer, so seemingly different from each other, occur in man in response to what is presumed to be a common carcinogenic stimulus.

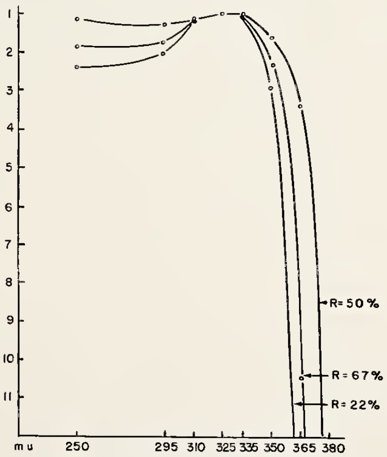
DISTRIBUTION OF SOLAR ULTRAVIOLET RADIATION AND TUMORS ON THE HEAD

Frederick Urbach

In our laboratory, we have studied the distribution of UV on models of the human head exposed to sunlight with the use of a chemical dosimeter system consisting of a halogenated hydrocarbon and an indicator dye. Irradiation of the hydrocarbon with UV light produces halogen-free radicals, which remove a proton from any available hydrogen donor to form acid and thereby cause a color change in the indicator dye (methyl yellow). There is a linear relationship between the dose of UVR and the development of the color. The wavelength response of the system shown in text-figure 13 is fairly constant from 250 to 330 nm; the sensitivity then falls rather abruptly, reaching about one-twelfth of the maximum at 365 nm. The system is insensitive to visible wavelengths. This spectral response does not correspond closely with the CIE curve (text-fig. 2) but is assumed to be a reasonable approximation for the purpose.

Models of the human head coated with the dosimeter system dissolved in a paraffin base were exposed to sunlight on clear days on the roof of the Philadelphia Skin and Cancer Hospital. With the head in a vertical position, the top of the head, nose, tops of the ears, lower lip, and neck received most of the UV recorded by the dosimeter. In contrast, the orbital area, the nasolabial fold, the upper lip, the submental area of the neck, and the postauricular areas received less than 18% of the intensity of UVR incident on the top of the head.

The large proportion of UV-B scattered back from the sky has been discussed by Schulze. By using opaque shields, one can illustrate the importance of this factor in the distribution on the head. For example, with the sun at 65°, the interposition of an opaque disk, which cuts off the direct radiation from the sun and only a small surrounding portion of the sky, reduced the UVR incident on the top of the head by about 40%. Under the same conditions but with a shield arranged to eliminate most of the sky as well as the direct radiation from the sun, the total UVR recorded by the dosimeter was reduced to less than 10% of the total.



TEXT-FIGURE 13.—Wavelength response of chemical dosimeter system used in studies of the distribution of solar UVR on the head. Relative sensitivity is expressed as the energy ratio necessary to produce a color change at a given wavelength.

Earth, grass, and water reflected only about 5–6% of the incident radiation detected by the dosimeter, whereas sand reflected almost as much as aluminum foil. When we placed the head above a sand surface, areas that were shielded from the sun and sky by anatomical features received a good deal of UVR; under these conditions, we reduced the total radiation only about 10% by cutting out the direct radiation from the sun but retaining that from the sky.

It is clear that the distribution of UV on the head is influenced by a number of factors that add to the complexity of the problem of relating sunlight to cancer incidence. Nevertheless, to attempt to correlate the topographical distribution of types of skin cancer with the picture obtained with the dosimeter and model heads is interesting. This was done with data on the location of basal cell and squamous cell carcinomas obtained from records at the Tumor Clinic of the Philadelphia Skin and Cancer Hospital. An excellent correlation was found for squamous cell carcinomas, which were distributed on those areas of the head that received the most UVR according to the studies with the dosimeter and model. In contrast, about 40% of basal carcinomas were found on areas that receive less than 20% of the maximum radiation measured at the top of the head. A similar distribution of cancer types was reported by Magnusson (*26*) in 1935. This correlation suggests that UVR is a major cause of squamous carcinoma in man, whereas some additional factor operates in the induction of basal cell carcinomas.

Prevalence studies that indicate a considerable variation of the ratio of squamous cell to basal cell carcinomas in different parts of the world may be interpreted to show that the prevalence of squamous cell cancers is relatively higher in areas where there is greater insolation (*1*).

Possible evidence of a genetic factor has been culled from medical records of the Philadelphia Skin and Cancer Hospital that show that 46% of the patients with skin cancer were either born in Ireland or were of Irish extraction. Probably this phenomenon is one of selection. Those patients most prone to develop skin cancer with the least amount of exposure to UVR settled in an area that receives small amounts of this type of radiation. These observations agree with the observations of Silverstone, who also noted a predilection for people of Celtic descent to develop skin cancer, probably because they burn and do not tan well. It was pointed out that women have considerably less squamous cell carcinoma than men, primarily because two of the most commonly affected sites (i.e., the ears and neck) are shielded by hair. This observation was clearly established in relation to senile keratosis in Silverstone's studies.

EVIDENCE FROM ANIMAL EXPERIMENTS REGARDING SITE AND CUMULATIVE ACTION IN CARCINOGENESIS

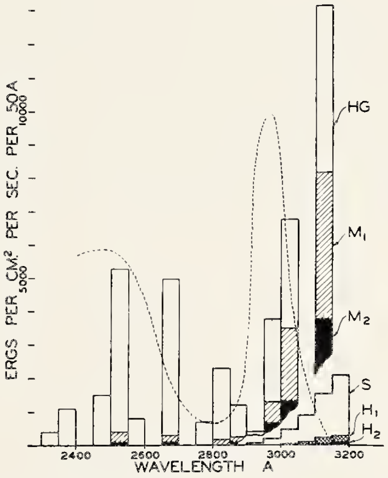
Harold F. Blum

Results of experiments with laboratory animals may have bearing on some of the problems just discussed. Some years ago in our laboratory, we found that tumors produced in the skin of strain A albino mice given repeated doses of UVR from an intermediate pressure mercury arc were predominantly dermal in origin (*20, 27*). Most of these were spindle cell sarcomas, with a number of hemangioendotheliomas and 1 osteochondrosarcoma; many were also epidermal carcinomas. This distribution of types is, of course, different from that found in human skin, in which epidermal carcinomas predominate among malignant tumors and sarcomas are rare. The apparent paradox seemed explainable on the basis of different amounts of UVR reaching the tissues in the two instances, since, other things being equal, it must be assumed that the chance of inducing cancer in a given tissue is related to the amount of UVR absorbed. Since human epidermis is much thicker than that of the mouse, we would expect that more UVR would be absorbed in the former (which would account for the high incidence of epidermal tumors in man) and that more radiation would pass through the epidermis of the mouse (which would account for the high incidence of dermal tumors in mice).

Measurements of the spectral transmission of UVR were undertaken (*28*) that gave support to this argument. These showed that much of the carcinogenic UVR from the mercury arc we were using passed through the mouse epidermis to reach the dermis; comparable measurements on samples of human epidermis indicated, as did earlier studies (*29*), that most of the UV-B of sunlight is absorbed in the epidermis. An idea of the spectral relationships may be gained from text-figure 14, which compares the incident radiation and the amount passing through the epidermis. Exact comparison of the radiant energies concerned in the two situations is not feasible, and the quantitative relationships indicated in the figure should be regarded only as approximations. Nevertheless, our experiments clearly demonstrated that the relative amount of UVR reaching the dermis of the mice was much greater than that which reaches the dermis of men exposed to sunlight.

Following this lead, we set up an experiment using low pressure mercury arcs emitting chiefly the 254-nm line, which is absorbed principally in the epidermis and (if our reasoning is correct) should produce a high incidence of epidermal tumors. An epidemic ended this experiment before conclusive results could be obtained; however, a few carcinomas in situ but no sarcomas were found (*30*). Later, Kelner and Taft (*31*) obtained comparable results with Swiss mice: a high proportion of spindle cell sarcomas with an intermediate pressure mercury arc but only epidermal carcinomas with a low pressure mercury arc. In experiments with strain A mice and low pressure arcs, I likewise found only tumors of epidermal origin. Thus the findings for both strain A and the closely related Swiss mice demonstrate that the kind of tumor is a function of the UVR absorbed in a particular tissue. This need not be the only determining factor, however.

In discussing the distribution of tumor types, we wrote in 1942: "It is concluded that ultraviolet radiation may induce tumors in a variety of tissues depending upon their susceptibilities and limited by the penetration of the radiation" (*26*). Even then we were aware that specific but unanalyzed characteristics of the tissue, called "susceptibility" for want of a better term, need to be taken into account as well as the amount of radiation absorbed. One of the things that led us to this idea was that the ratio of carcinomas to sarcomas varied markedly with the interval between doses but not with the dose. Studies on hairless mice with the intermediate pres-



TEXT-FIGURE 14.—Radiation reaching the dermis of the mouse under the experimental conditions and of man exposed to sunlight. Rectangles (HG) represent the energy of the intermediate pressure arc incident on skin; in most cases the 50 A.U. (5 nm) base of the *rectangle* includes only one line of the arc. The fraction of the radiation passing through the epidermis is indicated by: *solid rectangle* M_1 for unexposed mice; *shaded rectangle* M_2 for mice that have been repeatedly irradiated. S represents UV-B of sunlight; amounts of this radiation passing through the epidermis of the untanned and tanned skin are indicated by H_1 and H_2, respectively. *Dotted line* is the erythema spectrum. [Reproduced from (*28*).]

sure arc indicated that principally epidermal carcinomas are induced (*32*), but this is not surprising, since the skin of the hairless mice must differ optically from that of the ordinary albino and genetic differences may determine different susceptibilities of the tissues. It should be pointed out that observation of one type of tumor to the exclusion of another does not necessarily mean that carcinogenesis is taking place only in that particular tissue. If cancers are developing concurrently in different tissues, those that develop most rapidly will appear first and constitute the highest incidence; some may never reach observable dimensions within the lifetime of the animals (*20, 33*). Both the susceptibility of the tissue and the amount of radiation absorbed in it will affect the rate of development of the tumors, and other factors may be present as well.

We may suppose that both the amount of radiation absorbed in a given tissue and its specific susceptibility depend on genetic factors and that differences in incidence and distribution types among human strains, as indicated by Urbach and Silverstone and most clearly demonstrated in the comparison of incidences for Negroes and for white-skinned people, may reflect either the transmission properties of the skin or specific tissue susceptibilities, or both. We are not presently in a position to evaluate either of these factors, both of which could be genetically based. Transmission of UV light would seem on first consideration to be the easier to determine, but the optics of the skin are complex (scattering is particularly difficult to take into account), and it is unlikely that those few measurements made thus far are free from error.

Our experiments indicate that the effect of repeated doses of UV light is cumulative in carcinogenesis, and Epstein and Epstein have demonstrated that UV light and chemical carcinogenesis may be additive (*34*). Cumulative action is difficult to reconcile with concepts involving separate periods of cancer induction and growth and with the idea of a precancerous state (*27, 33*). In light of this evidence, is it not reasonable to assume that the so-called precancerous lesions of human skin are concurrent results of the action of UV light but with no direct sequential relationship? That is, the supposedly precancerous lesion does not become a cancer, but a cancer is likely to appear in the same locus. Statistical evidence of the independence of the two kinds of lesion was presented by Carmichael (*35*) but his analysis of the data has been challenged (*36*).

PART IV

METHODS FOR EPIDEMIOLOGIC STUDY

A. Lilienfeld

With epidemiologic methods, one looks for a statistical association between possible population characteristics and the disease, from which are derived biologic inferences. From the pattern of statistical associations, one derives leads to etiologic hypotheses. With the results from laboratory investigations, the inferences derived from epidemiologic studies may well provide information on the cause(s) of a particular phenomenon.

Statistical associations are usually derived in two major ways: 1) from the study of group characteristics and 2) from the study of the individual characteristics. As far as group characteristics are concerned in the study of skin cancer, several ways may be used to approach the study. One may record mortality and morbidity data; this requires an adequate reporting system. From information presented at this Conference, it appears that such studies could be done only with great difficulty in most parts of the world. Secondly, it is possible to study group characteristics by means of special population surveys. The studies of Silverstone and Gordon are fine examples of this procedure. In such studies, one attempts to measure the average exposure to UVR of a group and relate this to the incidence of skin cancer. Usually the groups being compared are in different geographical areas. One of the problems in such a comparison results from the fact that other variables which may influence the frequency of skin cancer have to be controlled. This may be quite difficult, since a decision must be reached as to which variables are important. Some of the important variables in skin cancer are age, ethnic background, sex, socioeconomic class, and occupation. The existing information clearly suggests that UVR is a causative factor in skin cancer. That the determination of these relationships in epidemiologic studies is consistent with the biologic hypothesis should also be noted.

In studies of individual characteristics, one attempts to determine the relationship of the characteristic to the disease in a single patient. Thus one is interested in determining what proportion of patients with and without the disease have a certain characteristic. This can be done by a cross-sectional study, e.g., the relationship of sunlight to senile keratoses in Australia. Another approach is the retrospective investigation in which one compares the history of exposure of patients with the disease to similar histories in patients without the disease. Retrospective studies may be biased by a "selective" memory and the problem of obtaining an adequate history of the disease under study. The third approach is the prospective one; in this type, individuals exposed to possible etiologic factors are selected from the population as well as a group of those who are not so exposed. Both groups are followed to determine the frequency with which each group develops the disease subsequent to the exposure. This is by far the best approach to the problem, although it is difficult, expensive, and usually requires large population samples. It was suggested that this type of study could perhaps be done with albinos.

In the course of these discussions, various suggestions have been made as to the possibility of adopting some sort of general index for the effects of lifetime cumulative exposure to UVR. Histopathologic changes in exposed and unexposed areas of the body could be studied, but it was pointed out that criteria for aging effects and other spontaneous changes would have to be developed and this would be difficult. The possibility of obtaining skin autopsy or biopsy specimens from lifetime residents in an area and comparing these with similar specimens from patients with skin cancer was proposed. Steigleder has suggested that it might be possible to compare biopsy specimens from the same area in different patients in the same age group and to look particularly at the pattern of collagen by historadiography. Van Scott suggested that the height of the dermal papillae and the state and number of blood vessels might be used as an index of UV exposure.

Based on these and other discussions, the following study is proposed: 1) Select population samples in communities at different latitudes; 2) do some histologic comparisons at the beginning of the study as well as obtain information on such characteristics as age, sex, ethnic background, etc.; 3) follow these population samples for 5 years, during which time measure climatic change, UV exposure, etc., and the frequency of the development of skin cancer in these groups. It would then be possible to analyze the relationship of the frequency of skin cancer to all the variables on which information was obtained. It was pointed out that the existing data on the relationship of skin cancer to UV light is based mainly on group characteristics and, although this suggested an appropriate hypothesis, adequate confirmation on epidemiologic grounds is not available. However, in favor of the hypothesis are the experimental studies of cancer production by UVR in animals.

SKIN CANCER IN QUEENSLAND, AUSTRALIA

Harold Silverstone

Epidemiologic studies of skin cancer and keratoses were performed during 1961 to 1963 in three climatically different areas of Queensland. These may be identified on the accompanying map (text-fig. 15: a dry tropical area around Richmond; a wet tropical area centering around Cardwell; and the subtropical coastal region of Caboolture). The composition of the population and manner of life differ for these areas as well as the climate. All the people 21 years of age and over who have lived all their lives or for 30 years or more in these areas were contacted. About 85% of possible respondents cooperated.

In all, 2,227 persons were examined for skin lesions; past histories were checked when possible, and a questionnaire was completed that gave information on age, residential history, occupation, clothing, habits, complexion, ancestry, and liability to sunburning. The incidence data, summarized in text-figure 16, reveal that the prevalence rates for the skin lesions studied were high.

In attempting to interpret these findings, Silverstone and Gordon (*37*) arrived at the following hypotheses presented mainly in the hope that further discussion will be promoted:

1) The prevalence of both types of lesion studied rises with increasing total dosage of UV-B from the sun; a different rate of increase holds for each type of lesion.

2) Genetic background is an important factor, in a broad sense, in the production of susceptibility to keratosis and skin cancers; e.g., susceptibility in those of Irish ancestry.

3) Among people of similar genetic stock, there is variation from individual to individual in the degree of susceptibility to skin lesions.

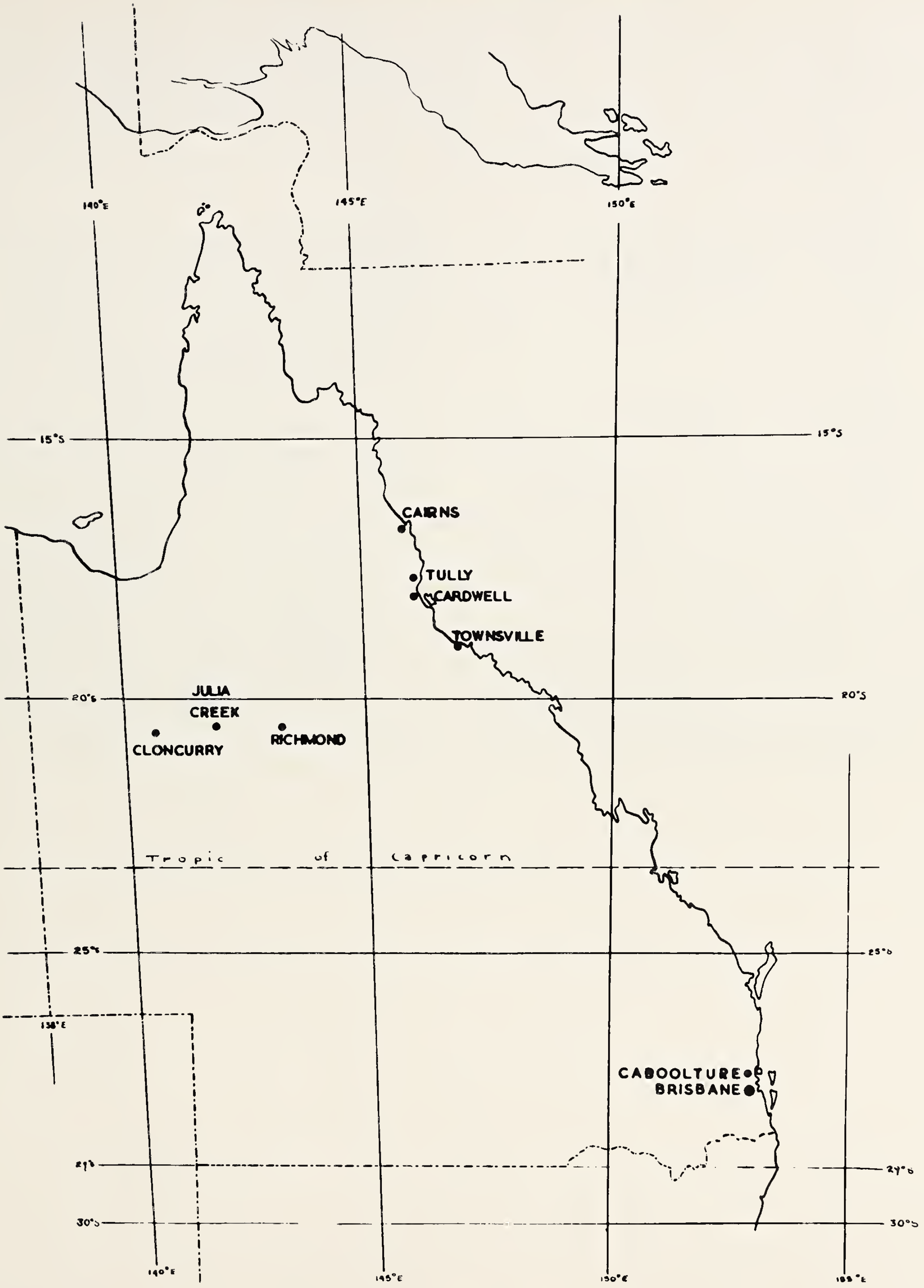
4) The particular type of lesion encountered depends on: *a)* the anatomical site, *b)* the dose rate (intensity) of the UVR, and *c)* the intervals between successive doses of UVR.

These three factors are not mutually independent. For example, the dose rate at any particular anatomical site depends on the angle of the sun's rays, whereas intervals between successive exposures depend on variations in protective clothing, etc. Again, the interval between doses of radiation of a given dose rate will depend on the particular dose rate concerned. Due to continual fluctuations in the level of UV-B from the sun, the intervals between periods of high-level radiation will be longer than those of low-level radiation, in contradistinction to the total amount of radiation received without consideration of the time in which it was received.

5) It is likely that basal cell carcinomas are associated with higher dose rate of UVR (received ipso facto at more widely separated intervals), whereas squamous cell carcinomas and keratosis are associated with lower dose rates and more frequent exposure at such rates. This hypothesis might account for the increase in the ratio of basal to squamous cell carcinomas in the "sunnier" latitudes, as well as some of the variation in this ratio among different anatomical sites and between the two sexes in the same geographical region.

6) Data showing that the average number of lesions per person is largely independent of the geographical region (and as far as the face is concerned, even independent of sex) suggest that susceptibility exists only in certain small separated areas (patches) of skin rather than uniformly over the skin as a whole. Once a certain total dosage of UVR has been received at a given anatomical site, these susceptible patches will develop lesions in quick succession.

7) Certain similarities in the anatomical pattern of the distribution of keratoses and squamous cell carcinomas could be a consequence of hypotheses 4 and 5 without the implication that keratoses are premalignant with respect to squamous cell carcinomas.


140°E
145°E
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15°S
CAIRNS
TULLY
CARDWELL
TOWNSVILLE
20°S
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JULIA CREEK
CLONCURRY
RICHMOND
Tropic of Capricorn
25°S
25°S
138°E
CABOOLTURE
BRISBANE
29°S
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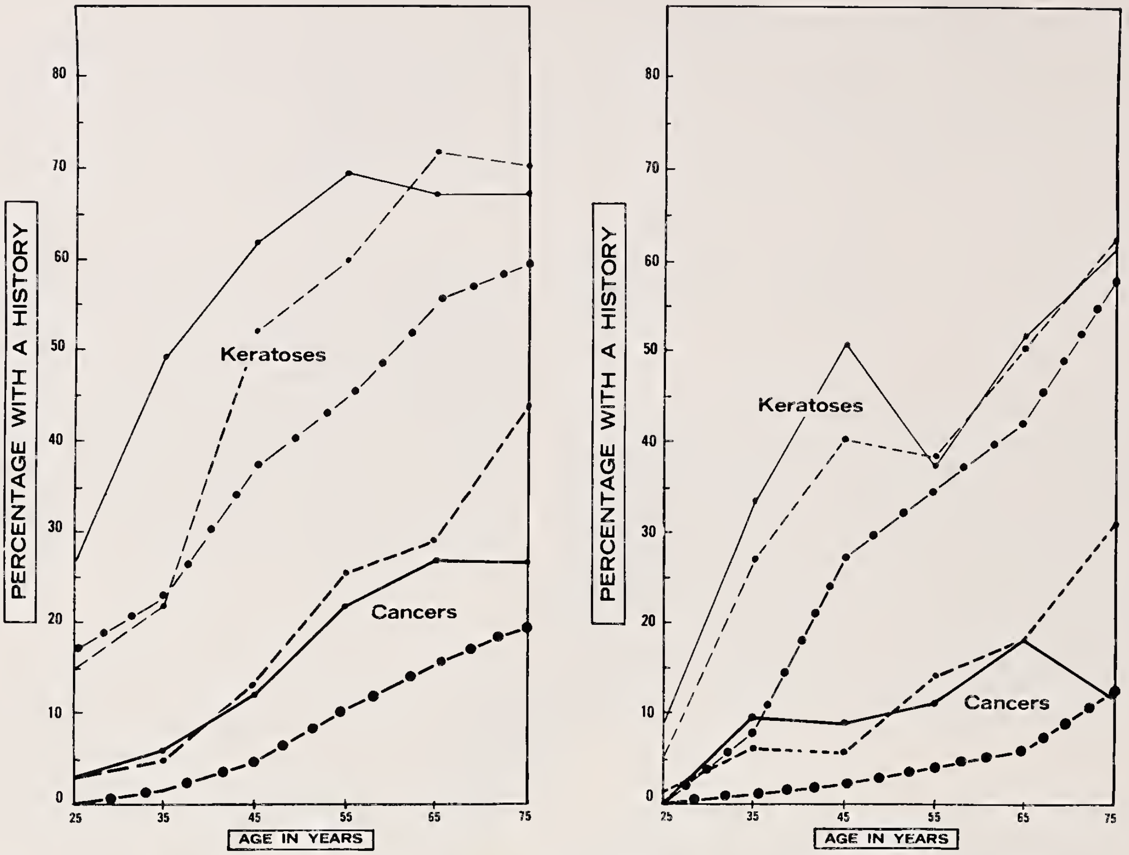
145°E
150°E
155°E

TEXT-FIGURE 15.—Map of the area in Queensland in which incidence survey was done.



Text-figure 16.—Summary of data collected in survey. *Left*=males *right* = females; ——— = Richmond, - - - - = Cardwell, and ● – ● – ● = Caboolture.

SKIN CANCER IN THE TRANSVAAL, SOUTH AFRICA

George H. Findlay

This report is based on 8.5 years of hospital practice in the city of Pretoria, which lies in the middle of the Transvaal at latitude 26° S. Of the 1.5 million white residents of the Transvaal, 1 million live south and the balance north of Pretoria. The 13,300 consecutive patients seen (9,000 private and 4,000 clinic) came from an area extending from 24° S to 28° S; 50 and 1.9% of the white population of Pretoria and northern Transvaal, respectively.

In this population were 446 patients with basal cell carcinoma (2.8% of all private and 4.5% of all clinic patients). The difference between private and clinic patients is probably due to prevalence of older people in the clinic population. The male to female ratio was 2:1. Among the male clinic population, the major incidence was between 70 and 75 years; 50% were over 65. Among the female clinic patients, the peak was between 60 and 65 years, with 50% over 60. In private patients, the peak incidence occurred at 60 years of age in men and at 50 in women. A rough calculation of incidence showed that basal cell cancer occurred in about 110/100,000 patients. The ratio of tumor types differed for clinic and private patients, as indicated in table 4.

TABLE 4.—*Ratio of incidence of tumor types*

Patients	Basal cell carcinoma Squamous cell carcinoma	Basal cell carcinoma Senile keratosis
Clinic	2.5–3	1
Private	6	1

The ratio for clinic patients is about the same as those reported from the Prince Albert Hospital, Sydney, Australia (*38*). Since the clinic patients tend to be older than the private patients, the table suggests that there is a greater increase in squamous cell cancer than basal cell cancer with age.

Oettle (*39*) has reported a death rate from skin cancer in South Africa of 3.5 and 1.45/100,000 for males and females, respectively. In checking carefully into the causes of death reported in cases of skin cancer in the Transvaal, I found that of 33 patients having carcinoma of the skin, this type was the cause of death in only 16; 6 patients had skin cancer present at the time of death, and the rest were either wrongly classified or the skin cancer was a metastatic or a sarcomatous lesion. Thus Oettle may have overestimated the death rate by approximately one-third.

Basal cell carcinomas hardly ever occur in Bantu Negroes (I have seen only one basal cell carcinoma in a Negro Bantu). However, an occasional hamartoma may be seen.

SKIN CANCER IN ENGLAND

R. Douglas Sweet

About 6 months ago (late 1963), a detailed survey of the incidence of all types of skin cancer was begun in four contrasting areas in England. The results will be studied at the end of a complete year and if differences are found to exist in the incidence of skin cancer in these areas, a full social and occupational survey will be done. Meanwhile for the purpose of this conference, an effort was made in the four areas in England where cancer registration was considered most complete to find out retrospectively the incidence of basal and squamous cell carcinomas during the years 1959 to 1962.

The areas chosen were the western two-thirds of the rural and seagirt county of Cornwall, the seaport of Plymouth, and the country extending about 50 miles east, north, and west. The population of this area is about half urban and half rural at a latitude of approximately 50° N; the seaport city of Bristol is 52° N and the heavily industrial city of Sheffield a little north of 53° N.

In each of the four areas, we detected about 1.5 basal cell carcinomas in males to 1 in females; a similar ratio in squamous cell carcinomas was 2.5 to 1. However, the incidence varied considerably. In Cornwall, approximately 40 basal cell carcinomas per 100,000 of population were detected annually, in Plymouth and Bristol about 30, and in Sheffield about 15/100,000. The differences were even more marked with squamous cell carcinomas; about 11/100,000 occurred in Cornwall, 9 in and around Plymouth, 6 in Bristol, and less than 1 in Sheffield.

The relative frequency is perhaps more easily demonstrated by giving the ratio of incidence of basal to squamous cell carcinomas in the four areas. In Cornwall, this works out at 3.5 to 1, in Plymouth about 4 to 1, Bristol 6 to 1, and Sheffield 20 to 1. Another fact that we learned from this retrospective study was that basal cell carcinomas appear to develop and peak earlier in life, whereas squamous cell carcinomas begin to appear later, and the incidence continues to rise with age despite the declining numbers in successive age groups.

This topographical distribution seems to match remarkably well that found by Urbach in his Philadelphia study, and the ages of onset, though relatively older, parallel Silverstone's findings in Queensland.

BEARING OF ANIMAL EXPERIMENTS ON THE INTERPRETATION OF EPIDEMIOLOGIC FINDINGS

Harold F. Blum

Silverstone and Gordon's hypotheses fit in numerous ways with interpretations of the results of laboratory experiments on albino mice [discussed at length elsewhere (*20*)] and can only be reviewed briefly here.

The quantitative relationships when dosage is continued until the tumors appear may be expressed by

$$\log \frac{V_d}{v_o} = \frac{bkd\,(t_d - t_o)^2}{2i(1-a/i)} \qquad (1)$$

where V_o is the volume of the tumor at the time of the first dose t_o, and V_d the volume at the time of tumor appearance t_d; D is the size of the individual dose, i is the interval between doses, and a, b, and k are constants.

The volume of the tumor (V_d) at the time of appearance is approximately the same in all cases, and if we assume that the tumor always begins to grow from a given initial volume (V_o), the left-hand member of (1) becomes constant. To a close approximation,

$$\frac{D\,t_d^2}{i} = \text{constant} \qquad (2)$$

The observed time squared relationship may be explained on the basis that growth rate of the tumor is accelerated by each dose.

We see from equation 2 that the time to appearance of tumors is inversely related to the size of the individual doses and directly to the interval between doses, which would seem to fit with Silverstone and Gordon's suggestions. Acceleration of development would account nicely for the rapid sequence of appearance of tumors in the same person. The idea of localized accumulation of effect (patches) is also suggested by some of our experimental observations (*20, 33*).

The time squared relationship indicates a cumulative process, as do other aspects of the evidence, particularly in those experiments when the dosage was stopped before the appearance of tumors, with a resultant delay in their appearance. The relationship between continued and discontinued dosage is described by

$$b(t_d - t_1)^2 = t_1\, t_2 \qquad (3)$$

where t_1 and t_2 are, respectively, the time during which the doses were given and the subsequent time until the tumor appears ($t_1 < t_d$; $t_d < t_2 + t_1$). This is the kind of relationship that would be expected if growth of the tumor were accelerated by the doses given during t_1 and afterwards continued at the rate reached at the end of that time.

All these relationships are explainable when a carcinogenic process continues throughout the development of the tumor but are difficult to explain when a precancerous condition changes in character to become a cancerous one. As was pointed out above, it seems more likely that keratoses and skin cancer appear in the regions of the skin that may be more susceptible or may have received more dosage than that the former is a precursor of the latter.

What has been stated earlier about tissue susceptibility and penetration of UV light into the skin would seem applicable to Silverstone and Gordon's ideas.

Extrapolation from the laboratory to the field is risky, particularly in this instance when the variability of the suspected provoking agent (UV-B) is so great. In a general way, our present laboratory findings do seem to predict phenomena observed in the field.

PART V

OTHER EFFECTS OF UV LIGHT THAT MAY BEAR ON THE SKIN CANCER PROBLEM

Up to this point, the discussion has dealt almost exclusively with chronic effects of UV light and cancer and other lesions that may be produced by repeated dosage with this agent. Acute effects that may follow a single exposure are common to our experience, i.e., in the form of sunburn, which is a rather complex response of normal skin. The relationships of these acute effects to the chronic are of considerable interest to us, since they have various aspects in common, at least some of which involve the basic action of UV light on living cells. The latter field of study has developed rapidly in recent years and cannot be adequately treated here, although its bearing on carcinogenesis by UV light must concern us.

Sunburn does not express itself as a single symptom but rather as a syndrome including: erythema, the formation and behavior of melanin pigment, and hyperplasia of the epidermis with thickening of both the viable layer and the corneum. Whether all these effects stem from a common photochemical reaction and thus have a common chromophore and action spectrum, or whether they involve different photochemical processes, chromophores, and action spectra could be debated. The problem may not be an easy one to resolve because of the complicated optical properties of the skin, which is far from a homogeneous system. The same general range of wavelengths causes erythema and the production of melanin pigment, although whether the action spectra are the same is not clear. The problem is also complicated by another effect, that of direct pigmentation, first described by Henschke and Schulze (56) and explained by them as oxidation of bleached pigment that has been previously formed and reduced to a leuko-form. The action spectrum for this effect is different from the erythema spectrum, extending through the longer UV into the visible ($\sim$ 300–440 nm).

ACTION SPECTRUM AND MECHANISM OF ERYTHEMA, AND ACTION SPECTRUM FOR CARCINOGENESIS

Ian A. Magnus

Using a large liquid prism monochromator, with a xenon arc as the source, we studied the action spectrum for erythema production in human skin. Two peaks of maximum reactivity were found at about 250 and 300 nm, and a somewhat variable minimum at 270 to 280 nm. The amplitude of the peaks and of the minimum varied considerably among the 26 persons studied. The position of the minimum agrees with the suggestion of Blum *(41)* that it is due to absorption by the overlying corneum, which is predominantly protein and has a maximum absorption at about 280 nm.

Various mechanisms have been proposed for erythema production, among others that of Mitchell *(42)*, who in 1938 suggested that in photolysis of protein in the epidermis, as a source of dilator substance the latent period between exposure and erythema appearance was due to the slow diffusion of the chemical mediator. If the Stokes-Einstein equation is applied, the molecular weight of the erythema-producing substance was estimated to be more than 1,000. A year later, Henschke and Schulze *(40)* suggested that damage to nucleic acid was a major factor in the production of erythema, and Claesson et al. *(43)* suggested that 5-hydroxytryptamine might be the cause.

van der Leun (personal communication to I. A. Magnus) measured skin temperature during erythema production and found that when the erythema appeared about 4 hours after a single moderately large dose of 300 nm radiation, the skin temperature did not rise (in fact, it declined slightly at first) until the end of 5 to 6 hours, i.e., about 1 to 2 hours after the skin had become distinctly red. A possible interpretation of this is that the temperature rise is due to dilation of small vessels (arterioles and venules) deeper than the capillaries which, although they dilate, do not increase skin temperature. This coincides with the idea that some substance formed more superficially diffuses first to the capillaries and only later to the deeper vessels. Calculations by van der Leun suggest that the chemical dilator may have a molecular weight of approximately 1,000; this is in agreement with the estimate of Mitchell *(42)*. The same type of calculation does not work well, however, when 250 nm are used, which suggests a different mediator or even a completely different mechanism, possibly a direct action of 250 nm in the dermis.

It was pointed out above that experiments on laboratory animals set the long-wavelength limit for carcinogenesis at about 320 nm, approximately the same as that for erythema production in human skin. Friedrich *(44)* and later Wetzel *(45)* attempted to study the action spectrum more carefully and exposed mice to monochromatic radiation obtained with a large quartz monochromator and mercury arc. The latter claimed that neoplasms were not produced by wavelengths of 280, 289, and 297 nm, and only a few with wavelengths of 302 and 313 nm. His yield of tumors was small, probably because the daily dosage was inadequate as compared with that used by other workers. These results seem compatible with the transmission of UV light by albino mouse skin indicated in text-figure 14, if the tumors were formed in the dermis (chiefly sarcomas) as found by Grady et al. *(27)* in mice irradiated with an intermediate pressure arc, and the wavelength distribution indicated in that figure. Wavelengths of 280 and 289 nm, which Wetzel found did not produce tumors, lie at a minimum of epidermal transmission, so that little of this radiation would reach the dermis; however, wavelengths of 297, 302, and 313 nm pass readily through the epidermis to be absorbed in the dermis. Kelner and Taft *(31)* produced tumors (all carcinomas) with a 254 nm wavelength, which is absorbed largely in the epidermis, and Blum now reports similar findings (p 151).

In studies now under way in my laboratory, we are investigating more thoroughly the wavelength dependence of carcinogenesis in albino mice and are using large monochromators with xenon arc as the source.

Xeroderma pigmentosum is a condition of the skin in which senile and neoplastic changes occur in childhood; the cancers are predominantly squamous cell carcinoma. The disease, inherited as a recessive genetic character and hence rare, is no doubt exacerbated by UV light. Using our irradiation monochromator, we found that the erythema spectrum of 5 patients did not differ significantly from that of normal skin. However, in 4 the erythema produced by wavelength 300 nm at dosages at or near the threshold appeared much later than normal, e.g., only at the end of 36 to 48 hours. Similar observations were made by Rottier *(46)*. On the other hand, the erythema appeared after about the same latent period as in normal persons with 250 nm.

DIRECT PIGMENTATION

(Based on a report by G. H. Findlay with discussion by other members)

Direct pigmentation differs widely in its expression among individual skins. In their original description, Henschke and Schulze (*40*) stated that they could not produce the phenomenon in 13% of the group studied. Persons exposed to the sun at a 50-degree solar angle developed direct pigmentation only; if they were exposed for over 40 minutes, both erythema and pigmentation appeared. Direct pigmentation occurs rapidly, reaching its maximum almost immediately, and usually disappears rapidly, so that at the end of 10 days it is barely perceptible, although in some persons, signs of direct pigmentation may remain at the end of 100 days. Magnus stated that he had not seen direct pigmentation provoked with monochromatic light from his monochromator last more than a few hours, and Findlay made a similar observation. There is perhaps some question of criteria of observation used in various cases. Findlay stated that it is possible to produce direct pigmentation in 100% of African Bantu.

The dependence of direct pigmentation on O_2 was demonstrated by Henschke and Schulze (*40*); the anoxia produced by a sphygmomanometer cuff inflated to above venous pressure was sufficient to prevent its appearance. Findlay stated that injection of adrenalin or procaine into Bantu skin followed by irradiation with UV light produced a blanched spot in the center of the irradiated area that looked like a lymphatic angiogram. Pigment darkening occurred except where this vasoconstriction had occurred. This again indicates the dependence of direct pigmentation on O_2 tension. Urbach pointed out that extreme anoxia is difficult to produce in the skin, and that the success of the methods described indicates that direct pigmentation must be highly sensitive to oxygen lack.

It is generally accepted that in contrast to direct pigmentation, erythema and primary formation of pigment are independent of O_2, but this may not be as clear-cut a distinction as has been supposed. Lacassagne and Latarjet (*47*) found that when newborn mice were irradiated in N_2 with controls irradiated in O_2; erythema was O_2 dependent, whereas a histologically demonstrated inflammatory reaction was not. It is also generally assumed that the action of UV light on living cells is independent of O_2, but Cook and Blum (*48*) found that this was not true for all wavelengths that produce hemolysis of washed erythrocytes. In the UV region, hemolysis was independent of O_2, and it is likely that proteins and lipids are involved, since no nucleic acid is in these cells. At about 180 nm, hemolysis was O_2 dependent and formation of O_3 was probably involved; at approximately 365 nm, hemolysis was also O_2 dependent, but perhaps this was due to photooxidation by O_2 (photodynamic action), with free porphyrin acting as a chromophore. The possibility that photodynamic action (*49*) takes place to a limited extent in some normal skins may need to be considered with regard to the variability of the direct pigmentation phenomenon, since both free porphyrin and free riboflavin and possibly other substances could act as photosensitizers in the spectral region concerned. All in all, it would seem that the question of O_2 dependence of the action of UV light on skin would merit reexamination.

Findlay discussed pigmentation in the Bantu skin. Significant amounts of pigment are present in the basal layer of the stratum corneum, as can be shown histologically, but little pigment in the form of granules is recognizable in the stratum malpighii. The question arises as to how the melanin (presumably formed originally in the melanocytes) is shared between the daughter cells in division of the basal cells; glycogen granules diffuse and sediment, whereas the melanin granules do not. With regard to the stratum corneum, one may ask whether melanin could be present in a bleached form and not be recognized in the histologic sections. In the Bantu, the pigment is normally distributed as a supernuclear cap; this cap can be bleached with dithionite, and if the pigment is subsequently reoxidized with chromate, the entire cell is filled with melanin. Is the melanin present in some nonpolymerized form that might be demonstrated with the electron microscope?

In direct pigmentation, which occurs apparently in the basal cell layer, the perifollicular areas darken sooner than other parts of the epidermis. This may be an optical phenomenon, however, due to the superposition of the melanocytes as one looks down through the skin. With repeated exposures to UV-B, direct pigmentation becomes less reversible, and eventually there is permanent pigment darkening.

As a working hypothesis to explain pigment darkening, Findlay (*50*) proposes that the UV light decreases O_2 consumption in the cell and leaves more for the bleached melanin granules to react with and hence to darken. He cited, in support of this, experiments that showed doses of UV light comparable to those that elicit pigment darkening decrease the O_2 consumption of mitochondria.

PROBLEMS IN THE OPTICS OF EPIDERMIS

Farrington Daniels, Jr.

The optical properties of the skin have been investigated only generally, and the relationship between the fine structure of the complex epidermis and the absorption, reflection, and scatter of different wavelengths of radiation can only be described as unstudied problems. Part of the difficulty is that the techniques of histopathology: e.g., fixation, dehydration, cleaning, and mounting in balsam, are designed to make the specimen "clearer" by alteration of the refractive index of the medium in which the section is mounted.

We have little information on the dimensional changes of pieces of skin during processing. We do know that excised skin contracts as it is removed. Presumably, shrinking also occurs during fixation, dehydration, etc.; therefore, the most precise micrometer measurements we can make with a microscope have unknown accuracy in terms of the living state. Appropriate studies may have been made but they are not general knowledge. Precise dimensions are supplied by the electron microscopists, but again the dimensional changes in processing are not routinely discussed in papers in which the observed measurements are given with great precision. However, we must start with the information we have, as well as realize the need for better quantitative data.

Most of the computation and speculation about the effects of UVR on skin has assumed a homogeneous protein or nucleoprotein target. The new knowledge of subcellular organelles and other features of ultrastructures and molecular biology raise the possibility of specific localized targets for the initial UV effects.

The Stratum Corneum

The stratum corneum, a mechanically tough and chemically resistant layer, consists of flattened cells without nuclei that essentially have a cell membrane 1.25 nm thick as seen under the electron microscope. The flattened cells are joined by 20-nm desmosomes. The cells contain 10-nm round filaments, which do not stain with osmium, and are embedded in an electron-dense cement substance. The filaments lie horizontally to the surface, and show birefringence under the polarizing microscope.

According to Zelickson *(51)* the stratum corneum is usually 4 to 8 cells thick; the cells are flat and undulating with the long axes parallel to the surface.

Barer (*52*) states that for "simple" soluble proteins, the refractive index increases by 0.00185 for every 1% increase in concentration. Concentration must be expressed as mass per unit volume, not in terms of grams per 100 grams of solute. He studied living cells by immersion refractometry, using a phase contrast microscope as a null indicator, and beef albumin solutions of different concentrations as the immersion medium. The stratum corneum swells as it takes on water. Thus, it would appear that the optical properties of the stratum corneum may include wide variations in the refractive index and that these changes could be determined environmentally. If, as seems likely, the cementing substances and the keratin fibers have different water-binding properties, the relative refractive indices of the matrix and the fibers will change and affect refraction and scattering.

Thomson (*53*) showed that the stratum corneum of the Negro acts as a filter because of its melanin content and not because of greater thickness. Miescher (*54*) emphasized the same point in his classic article.

Although the stratum corneum cells are not densely filled with melanin granules, melanin is present and must play a role. Melanin granules and dust are in the range of particle size from 0.1 to 1.2 μ. Thus these particles are in the range of Mie scattering. Stacey (*55*) states that the Mie treatment of scattering is applicable to the scattering of spherical particles in the size range of 0.05 to 1.0 μ and to any difference in refractive index between the medium and the particle. When the particles are larger than 1/20 of the wavelength, they do not act as single point scatterers, and each point of the particle acts as a scattering point; hence phase differences in the scattered light may arise. Therefore, destructive interference that tends to be greatest at a 90-degree angle from the incident may occur. More scattering is seen in a forward direction than to the side in Mie scattering as compared with Rayleigh scattering. The theory of Rayleigh covers the scattering of small particles when the difference between the refractive index of the particles and the medium is small (Stacey).

The importance of the scattering function of melanin granules in the skin has not been determined, but under the darkfield microscope, melanin granules appear as bright spots of light.

The Stratum Granulosum

Beneath the stratum corneum is the stratum granulosum. In this area are few mitochondria, vesicles in the endoplasmic reticulum, melanin granules and smooth, thick-walled granules, sometimes called Odland bodies. The particular feature of this area at which the skin becomes keratinized are large electron-dense, irregularly shaped kerato–hyaline granules readily visible under the light microscope.

Basal and Prickle Cell Layers

The epidermis beneath the granular layer consists of basal and prickle cells of the keratinocyte series and melanocytes and Langerhans of the pigment cell series. Melanin granules are formed in melanocytes and transferred to keratinocytes.

In the living keratinocytes of the prickle and basal cell layers, the cells could be considered as a bag of protein solution but for greater precision should be considered in terms of the cytoplasmic content of protein, ribonucleic acid, vertically oriented keratin fibrils, melanin granules, and mitochondria, as well as the nucleus. The refractive index of these structures is unknown but can be estimated from particle size and density information.

The finding of Finsen that black pigment could protect the skin from UVR led to the oversimplified idea that melanin content explained UV protection. When it was found that some adaptation of the erythema threshold took place with repeated UV exposure in vitiliginous skin and that increased tolerance to UV exposure preceded the increase in melanin and declined before visible melanin decrease, a counter oversimplification arose: Melanin has no role in UV protection.

Another factor in the uncertainty about the function of melanin has been its concentration in the basal layer of the epidermis, whereas histologic evidence placed much of the epidermal UV damage in the upper half of the prickle cell layer, well above the site of melanin concentration. Attempts to explain this finding have included speculations about melanin as free radical traps.

I have recently reviewed, in much more detail than can be given here, the problems of short- and long-term adaptation to UVR (*56*).

It seems to me that the short-term loss of erythema response in vitiligo and albinism following UV exposure may be misleading and may represent a loss of target as well as a protective effect. During the first few days following sunburn, not only the stratum corneum but also the prickle cell layer is greatly thickened, and there is a heavy concentration of RNA in the cytoplasm that might make the regenerating epidermis a much more effective UV filter than the undamaged one.

Conclusion

These remarks may serve as an introduction to the problems of epidermal optics and indicate the need for much more quantitative information.

NATURAL PROTECTION AGAINST ULTRAVIOLET B

Harold F. Blum

The fact that Negroes sunburn less readily than white-skinned persons has led to widespread acceptance of the idea that the former are protected by the absorption of the sunburning radiation. Less generally recognized is the protection afforded by the corneum, which, because it is composed largely of protein, absorbs the UV-B; this absorption is greatly enhanced by scattering which effectively increases the pathway through which the rays must pass.

That exposure of the skin to UVR reduces sensitivity to subsequent exposure is common knowledge and generally attributed to the pigment that appears in the skin about the same time. Simultaneously, hyperplasia of the epidermis occurs and consequent thickening of the corneum that should contribute its share of protection. The latter was demonstrated and its significance recognized by A. C. Guillaume in 1926 (*57*); and quickly confirmed by Miescher (*58*), and by Lovasatti (*59*). For sometime after this, many thought that thickening of the corneum plays the predominant role in protecting human skin against sunburn. It had been demonstrated earlier that sensitivity of albino skin to UVR is reduced by exposure to that agent, and by reference to text-figure 14 one can see that this is the case for albino mouse skin; this evidence also indicates the importance of thickening of the corneum.

In accord with this idea, it was supposed that the insensitivity of the Negro to sunburn results from his possessing a thick corneum. This was confirmed by Thomson in 1955 (*53*), who at the same time upset the apple cart by showing that the Negro corneum is not only thicker but more opaque per unit thickness than is the skin of the light-complexioned people he studied. Both factors must be involved, and until we have more adequate measurements of the comparative thickness of skin exposed to the UV-B range, we cannot decide how much protection is to be attributed to the amount of melanin and how much to thickness of the corneum. The contention of Mackie and McGovern (*60*) that the thickness of the corneum is insignificant seems based on an error in their assumption that absorption varies linearly with thickness, instead of exponentially.

This uncertainty is of particular interest in the present context because of our general tendency to assume a close correlation between dark color of the skin and refractoriness to sunburn and skin cancer, without regard to other complicating factors which have been discussed previously. The generalization may be a reasonable one, but it is likely to lead to error if too closely applied to geographical distribution of skin cancer.

The notion that dark-skinned peoples are adapted to life in the tropics because their pigment protects them against harmful rays of the sun is an old one. Charles Darwin suggested this in 1871, tentatively linking the idea with natural selection; about the same time, M. Wedding reached a similar conclusion, basing this contention on his classical demonstration of the role of sunlight in buckwheat poisoning of domestic animals. In the light of present knowledge, both arguments seem irrelevant (*61*). Finsen's subsequent demonstration that india ink on the skin surface protected against sunburn may also have had its effects (*41*). Whatever the relative weight of these arguments in establishment of the notion that dark-skinned races inhabit the tropics because of their refractoriness to sunlight, it is now firmly fixed in the popular mind. Therefore, I suggest that you compare the map in text-figure 3, which purports to show geographical distribution of skin color, with distribution of UV-B shown in text-figures 4–6. The correlation is not very convincing.

ON THE MECHANISM OF RADIATION CARCINOGENESIS

Raymond Latarjet

In experimental carcinogenesis, only the two ends of the process are known as a rule; i.e., the time of first application of the radiation and the appearance of the tumor. In the intervening sequence of events, one must distinguish two steps: the malignant transformation of one or several cells and the clonal growth of these transformed cells to the observable tumor. Malignant transformation of a cell can be direct (mutation) or indirectly mediated by another agent (virus). Radiation may cause both types of transformation and may also allow the cancer cells to grow by clonal selection. The fact that radiation may thus intervene at several levels of the malignant process explains how difficult the present analysis is.

In the more detailed discussion of these possibilities, direct malignant transformation may be considered first. Both UV and ionizing radiations are known to be mutagenic. A difficulty in assuming that radiation initiates a carcinogenetic process by inducing a mutation lies in the fact that the mutated cell must survive to express its malignancy. Therefore, the change must be minute not to be lethal. Examples of such minute changes from radiation chemistry are transformations of one base into another; for example, cytosine to uracil, thymine to 5-hydroxymethyluracil. Change of a single base may alter the genetic code in such a way that one amino acid is synthesized or incorporated into a protein instead of another one. Such genetic change may not be lethal and may be progressive with continual radiation. However, the kinetics of cancer production by radiation with time and dose does not fit well with this simplest scheme of mutation.

An indirect pathway involving the participation of a virus is a possibility. In essence, a virus may induce the malignant transformation of a cell by incorporating its genome (or part of it) into that of the cell, according to a process similar to lysogenesis in bacteria. The role of radiation would be to favor such incorporation. For example, it could render the cell more competent for the viral genome. Conversely, it is most unlikely that the radiation may act by some kind of activation of the virus that would lead to viral production. Such viral production might kill the cell in which it takes place, as it does in irradiated lysogenic bacteria.

The case of viral transformation could be a particular one in a more general concept: A virus is a wonderful machine which nature has devised to inject a foreign nucleic acid into a cell. The efficiency of this machine may reach 100%. However, such a mechanism is not indispensable for the introduction of nucleic acids. The nucleic acid of the virus is still effective in pure form (less effective by a factor of 10^{-4}). Spontaneous transformation may occur in bacteria (as shown by Catlin and others). Therefore, cells spontaneously release DNA in such a form that other cells may incorporate them. Thus the DNA withstands the chemical and physical forces, and small parts of the molecule may transfer the genetic characteristics. He suggested that spontaneous release and incorporation of DNA may occur in a tissue. The question is whether radiation augments or initiates this phenomenon; there are isolated reports of instances suggesting it. Interferon production (small proteins which are virucidal) may be stimulated by inclusion of the foreign DNA in spontaneous fashion. (It may be pointed out that methylcholanthrene inhibits the synthesis of interferon.) Radiation damage increases the amount of release and absorption of RNA and DNA. This may be due to an effect on the cell membrane. It has been shown to occur in various bacteria, yeasts, and cells. Incorporation is also increased by radiation (e.g., the entry of viruses). A dose of 500 roentgens increases polyoma virus transformation in tissue culture by a factor of 3. Furthermore, it has been shown that in X-ray-produced leukemia in the mouse, the X-ray changes increase the ability of thymic cells to incorporate the virus. Thus radiation may 1) produce specific chemical changes in DNA, 2) increase the ability of foreign DNA to travel, and 3) decrease the ability of the tissue to cope with the traveling DNA. *Therefore, the malignant cell may not be the altered cell, but a cell nearby that accepts the altered DNA.* This agrees with Blum's idea of a "traveling" material and the sizes suggested fit reasonably well (Tem theory of Blum).

COMMENT

Harold F. Blum

In a general way, the ideas of Latarjet agree well with my own, but they express more explicitly the kind of changes that may take place in the transformation of normal cells into malignant ones. I may point out, however, that in our experiments, the intervening state of the tissue between the first application of UVR and the appearance of the tumor was probed by our discontinuing dosage at specific times. Analysis of the data obtained indicate quite clearly that the malignant transformation must be a gradual one. This is also supported by other aspects of our data, including rates at which the tumors are growing shortly after they appear. These are such as to preclude explanation in terms of either single mutation at the cellular level or "clonal selection." However, this does not rule out small intracellular alterations of the genetic code as suggested by Latarjet, provided that the effect of these is cumulative within the cell. I am sorry to say that my estimation of the size of the "Tem" (*20*), plausible as it seemed when I made it, seems to have dissolved into thin air with further analysis.

PHOTOREACTIVATION

The reversal of effects of UV-B on cells by the action of longer wavelength UV and visible radiation (wavelengths of 300–500 nm) is generally known as photoreactivation (also called photorecovery and photorestoration). First discovered in 1949 by Kelner, this phenomenon has now been demonstrated in various kinds of cells and may be considered to be generally distributed throughout the phyla. Rieck et al. (*62*) have shown that photoreactivation takes place in mouse skin, and although not yet demonstrated in human skin, it may safely be assumed to occur there. This action is interesting because the radiation that produces it is much more intense in sunlight and varies less with solar angle than UV-B. Thus it might have a considerable effect on carcinogenesis by sunlight and on the geographical distribution of skin cancer. The phenomenon was discussed only briefly at the conference; its basic aspects have been ably reviewed by Jagger (*63*).

PART VI

RECOMMENDATIONS FOR FURTHER STUDY

As a result of this conference, certain types of studies seem advisable.

Epidemiology Studies

Criteria should be established for the collection of data on the incidence of skin cancer so that pertinent information may be compared from different parts of the world. If feasible, these should include: 1) incidence of chronic skin lesions, malignant or nonmalignant; 2) type of lesions, preferably based on histopathologic examination; 3) anatomical position of lesions; 4) history of residence, with particular reference to exposure to sunlight and other environmental factors; 5) genetic information, e.g., ancestry, complexion history of skin cancer in close relatives, and history of residence of these relatives if possible.

Histopathology Studies

These should include not only studies of malignant tumors but of associated skin lesions. The study of histologic changes in skin with age should be pursued, including comparison of skin of exposed and unexposed areas.

Sunlight Measurements

Measurements of the UV portion of sunlight should be made at selected points on the earth, including: 1) detailed spectral studies comparable to those already done at Davos, Switzerland, and 2) development of portable field instruments for measuring the UV-B. These investigations should be standardized against measurements of the first type and performed simultaneously and at the same site.

Laboratory Studies

Research should continue on carcinogenesis by UV light with laboratory animals, with special emphasis on quantitative aspects; wavelength dependence; effect of dosage schedule, including interruption of schedule; and the additive effect of different carcinogens. Such studies should be done under standardized conditions, with theoretical interpretation in mind.

The studies of the optical properties of skin, with special regard to the penetration of UV light, should also be pursued.

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